Iraqi Journal of Science, 2024, Vol. 65, No. 4, pp: 1940-1947
DOI: 10.24996/ijs.2024.65.4.14

ISSN: 0067-2904

Effects of Oral Zinc Supplementation on Early Embryonic Development and Neonates of Aged Female Albino Mice

Amjed T. Al-Rudaini[1], Lina A. Salih[1], Saad S. Al-Dujaily[2]
[1]Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq
[2]Higher Institute for Infertility Diagnosis and Assisted Reproductive Technology, Al-Nahrain University, Baghdad, Iraq

Received: 6/2/2023 Accepted: 28/4/2023 Published: 30/4/2024

Abstract

Female reproductive aging is a normal phase of life that eventually leads to menopause and reproductive senescence. The current experimental investigation was intended to study zinc supplementation effects on early embryonic development and neonates in 26-28 weeks old female mice. In this investigation, 80 mature female mice were used that were divided into two groups of forty each. Two control groups received distilled water, while two Zn groups were given 0.115 mg/kg/day Zn orally daily during 2-3 estrous cycles. The female mice were all mated with adult males. Twenty female mice from both the control and Zn groups were euthanized by CO_2 gas inhalation, and then a longitudinal incision in the abdomen of each female mouse was performed and uterine horns and oviducts were detached from the body in order to obtain two-cell embryos. On the other hand, 20 pregnant mice from each group were permitted to complete their pregnancy until birth and then their neonates were collected for macroscopic examination. The findings showed that the Zn supplementation significantly ($P\leq0.01$) improved the mean embryonic development and the quality of 2-cell stage embryo grade A. Whereas, it decreased the mean embryonic development of 2-cell stage embryo grade D in aged female mice significantly ($P\leq0.01$) in comparison with aged control groups. Also, the count and quality of neonates from Zn-treated aged female mice enhanced significantly ($P\leq0.01$) as compared to non-treated aged female mice**.** It was concluded from these findings that the Zn can improve embryonic development and neonate count and quality in aged mice.

Keywords: Zinc, Aged mice, 2-cell stage embryo, Neonates.

تأثير التجريع الفموي بالزنك على التكوين الجنيني المبكر وحديثي الولادة في الفئران المسنة

امجد تركي الرديني[1]*, لينا صالح عبدالمطلب[1], سعد صالح الدجيلي[2]
[1]قسم علوم الحياة، كلية العلوم،جامعة بغداد، بغداد، العراق
[2] المعهد العالي لتشخيص العقم والتقنيات المساعدة على الانجاب، جامعة النهرين، بغداد، العراق

الخلاصة

يعد التقدم في السن لدى الإناث مرحلة طبيعية من الحياة تؤدي في النهاية إلى سن اليأس وعدم القدرة على الانجاب. هدف البحث التجريبي الحالي لبيان تأثير مكملات الزنك على التكوين الجنيني المبكر وحديثي

*Email amjed.torki@sc.uobaghdad.edu.iq

الولادة في الفئران الإناث المسنة (26–28 أسبوعًا).تضمنت هذه الدراسة 80 فأر أنثى بالغة، قسمت الحيوانات إلى مجموعتين، جرعت مجموعة السيطرة ماء مقطر ، بينما أعطيت مجموعةالعلاج بالزنك 0.115 مجم / كجم زنك عن طريق الفم خلال 2–3 دورات شبق.تزاوجت جميع الفئران الإناث مع ذكور بالغة. بعد مرور 24 ساعة من التزاوج تم الحصول على الاجنة في مرحلة خليتين من الامهات بعد تشريح 20 فأر من كل من مجموعتي السيطرة والعلاج بالزنك . من ناحية أخرى ، سُمح لـ 20 فئران حوامل من كل مجموعة بإكمال حملها حتى الولادة ، تم جمع الولادات لغرض الفحص المجهري.أظهرت النتائج بأن مكملات الزنك حسنت التكوين الجنيني المبكر وجودة الاجنة بمرحلة خليتين من الدرجة A وقللت من التكوين الجنيني للأجنة المكونة من خليتين من الدرجة D في إناث الفئران المسنة بشكل ملحوظ ($P \leq 0.01$) عند مقارنتها بمجموعة السيطرة . أيضًا ، تحسن عدد ونوعية حديثي الولادة من إناث الفئران المسنة المعالجة بالزنك بشكل ملحوظ ($P \leq 0.01$) مقارنة بمجموعة السيطرة. يستنتج من هذه النتائج أن الزنك يمكن أن يحسن التكوين الجنيني وعدد الولادات الحية ونوعيتها.

1. Introduction:

Physiologically, reproductive aging is a normal part of life for females which eventually leads to menopause and reproductive senescence, thus increasing maternal age results in pregnancy complications [1]. Henceforth, female aging is one of the most significant elements influencing human fertility. Female fertility gradually declines with aging due to a decrease in ovarian reserve and oocyte quality [2]. Additionally, low oocyte quality is a contributing factor in female infertility [3, 4]. The natural accumulation of free radicals like reactive oxygen species (ROS) with age could explain why females of advanced age have poor oocyte quality [5].

On the other hand, trace minerals are important for healthy and reproductive potential [6]. Zinc (Zn) is the second most prevalent trace mineral in the human body [7] and must be consumed on a regular basis as human body does not store it [8]. Zinc has a variety of diverse properties and functions as a hormone balancer in body, sexual health, as well as necessary to preserve the lining of the reproductive organs in excellent condition [8, 9]. Zinc also stimulates the formation of metallothioneins which are proteins that aid in the removal of hydroxyl radicals and sequestration of ROS [10].

Many antioxidant enzymes require Zn as a cofactor for effective antioxidant defense system function, for instance, superoxide dismutase that plays an important in the protection of DNA damages [11]. Zinc also protects cells from oxidative damage, assists to stabilize membranes, and inhibits the nicotinamide adenine dinucleotide phosphate oxidase enzyme (NADPH-Oxidase) [10] and has an essential role in immune system improvement [12].

Many studies have found that Zinc is important in fertilizability (13, 14). It is truly essential for the female reproductive system's health since the cells in this system differentiate and proliferate substantially, and these activities are Zn-dependent [15]. Zinc also regulates meiosis throughout oocyte maturation, including the maintenance and releases from the first and second meiotic arrest points also it is needed for the completion of meiosis [16]. In addition, it is important in female reproductive processes such as oogenesis, folliculogenesis, ovulation, oocyte maturation, fertilization and embryo development [17].

However, Zinc deficiency during fertilization and early cleavage can cause abnormal embryonic development [18]. However, the supplementation of Zn can induce a reduction in pregnancy complications [19]. Therefore, the present work aimed to investigate Zn oral

supplementation effects on early embryonic development, quality of early embryos and count and external morphology of neonates from aged female mice.

2. Materials and Methods:

This experiment was in accordance with Iraqi standards and was authorized by the local ethics council for animal research in the Biology Department, College of Science, University of Baghdad, under protocol number CSEC/1020/0053 in 2020.A total number of 80female albino Swiss mice (26-28 weeks old) were obtained form Al-Razi Center for Research and Medical Diagnostic Kit Production/Ministry of Industry and Minerals. The mice were housed in private owned facilities under a 12-hour light/dark cycles, with regular temperatures of 24 to 26°C and humidity of 30–40 %. They were provided access to food and water freely. The mice were divided into two groups at random (n=40): the control group was provided distilled water and the Zn group was administered 0.115 mg/kg/day of Zn (AMS, USA) daily for 2 to 3 estrous cycles. Male and female mice were mated, and then the female mice were examined for the presence of a vaginal plug which was considered an indication that mating had occurred. Day 0 of gestation was the day of the vaginal plug. After 24 hours following mating,1-day post coitum (d.p.c.), 20 female mice from both control and Zn groups were euthanized using carbon dioxide (CO_2) gas inhalation to harvest two-cell embryos for observation under an inverted microscope (TECHNO, Japan). On the other hand, 20 pregnant mice from each group were permitted to complete their pregnancy until birth, and their neonates were counted and examined under a stereomicroscope (Zeiss, Germany) for gross abnormalities [20].

Following CO_2 gas inhalation, each animal's uterine horns and oviducts were detached from the body and removed using sterile surgical equipment and gloves. The specimens were immediately placed in a culture plate supplied with phosphate-buffered saline (PBS; pH 7.3). The right and left uterine horns were dissected under a stereomicroscope (Zeiss, Germany). The technique of "flushing and dissection" was applied as described by [21, 22] to collect embryos from oviducts and uterine horns. The morphology of all retrieved early-stage, i.e., 2-cell, embryos was examined using an inverted microscope (TECHNO, Japan). The morphology of two-cell stage embryos was divided into four grades based on the grading system of Khalili and Anvari: A, B, C, and D [23].

3. Statistical Analysis

For data analysis, IBM SPSS for Windows, version 28 (SPSS Inc. Chicago, Illinois, United States) was applied. To determine statistical differences between means, one-way ANOVA was employed, and $P \leq 0.01$ was chosen to indicate significance [24].

4. Results

4.1 Estimation of Early Embryonic Development

Based on the findings in Figure 1, the number of embryonic development rate and the score of *in vivo* development of 2-cell stage embryos grade A of aged mice in the Zn group showed a significant ($P \leq 0.01$) rise compared to the control group. The results in the same figure reveal that there were no significant ($P > 0.01$) differences between the mean number of grade B embryos in the control and Zn groups. The findings also revealed that the mean number of grade C embryos in Zn and control groups was 0.20±0.04 and no significant ($P > 0.01$) variances were detected between them. At the same time, the mean number of grade D embryos showed a significant ($P \leq 0.01$) decrease as compared to control group. The grading of 2-cell embryos is shown in Figure2.

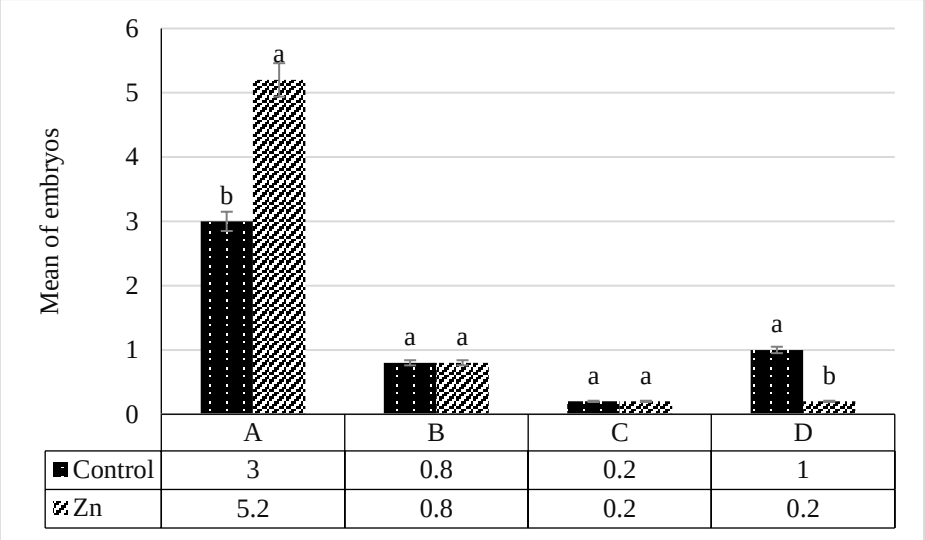

Figure1: Embryonic development rate of 2- cell stage embryo in aged female mice treated with Zn.

A: Grade A 2-cell stage embryo, B: Grade B 2-cell stage embryo, C: Grade C 2-cell stage embryo, D: Grade D 2-cell stage embryo, different small superscripts denote significant differences at P ≤0.01. n=20 replicates; Zn: Zinc group.

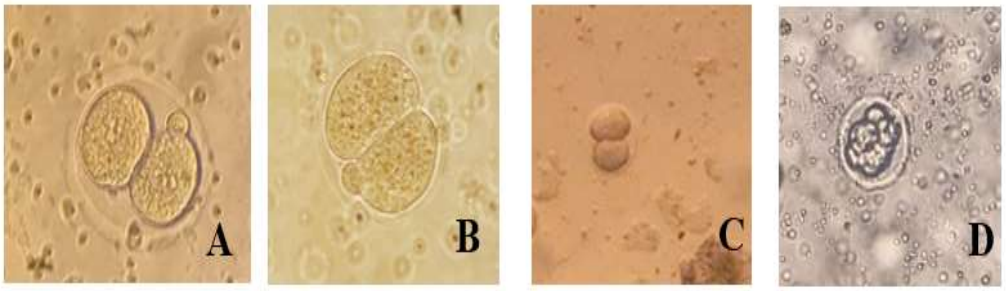

Figure 2: Shows grade A of good quality 2-cell stage embryo with equal size blastomeres (from Zinc group), grade B 2-cell stage embryo of slightly unequal size blastomeres (from Zinc group), grade C 2-cell stage embryo with unequal size blastomeres and fragmentation (from the control group), and grade D 2-cell stage embryo with unequal size blastomeres and sever fragmentation (from the control group), by inverted microscope. A and B magnification x 400; C and D magnification x 100.

4.2 Estimation of Neonates

The macroscopic examination of the neonates from both control and Zn groups showed no external malformations. The neonates had a C letter form, normal distinct facial characteristics, normal and prominent ear pinnae on both sides of the head, closed eyelids, a curved and prominent up straight tail, normal forelimbs and rear limbs with normal distinct digits (Figure3). The findings of the current study are illustrated in Figure4, where the mean of neonate count from aged female mice treated with 0.115 mg/kg/day of Zn can be seen when compared to the control group, increased considerably ($P \leq 0.01$).

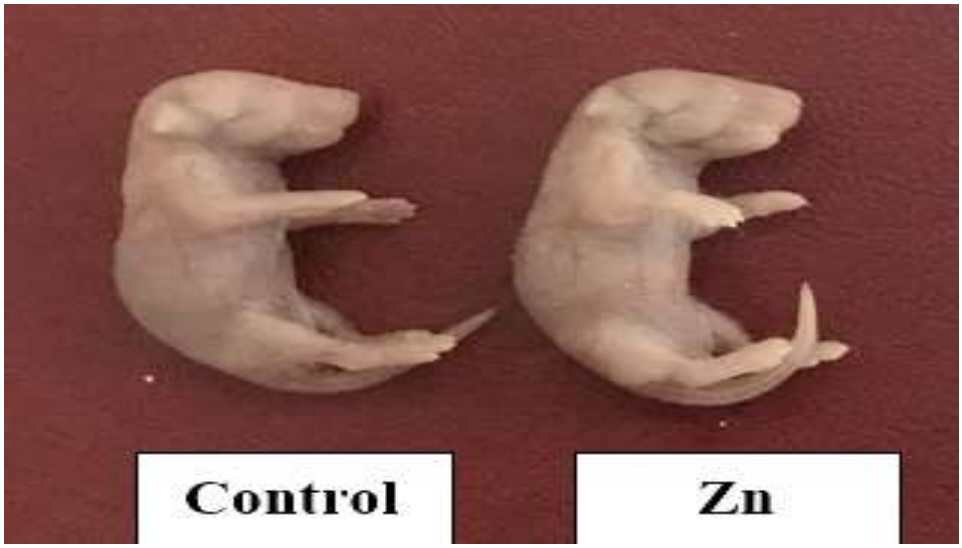

Figure 3: External view of the neonates of aged control and Zn-treated female mice.

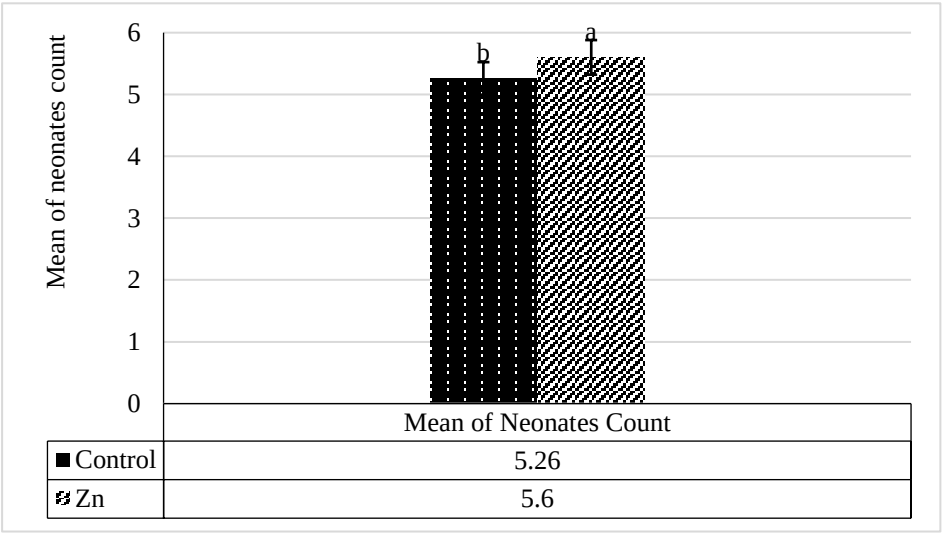

Figure 4: Mean of neonates count from aged female mice treated with Zn.

Different small superscripts denote significant differences at $P \leq 0.01$.
n=20 replicates, Zn: Zinc group

4. Discussion

The current study showed that the reproductive ability of 26-28 weeks old female mice that received 0.115 mg/kg/day of Zn orally enhanced in comparison with non-treated aged female mice. Zinc can promote follicle growth and development by maintaining prophase I arrest, establishing the phenotypic of cumulus cells, controlling steroid production, and DNA methylation. Furthermore, Zn can improve female reproduction by successfully resuming meiosis II of the oocyte during fertilization and transitioning from an oocyte to an embryo [25]. Zinc supplementation boosts the number of mature oocytes and the rate of early embryo development which is related to diminished ROS and increased intracellular Glutathione GSH levels [17]. It has been found that Zinc is an essential trace mineral for reproductive processes such as oocyte maturation and growth, fertilization, epigenetic programming, and subsequent embryonic, fetal, and placental development [26, 27].

Our results demonstrated that the rate of *in vivo* development of 2-cell stage embryos grade A of aged mice received Zn enhanced as compared to the control group, while the rate of *in vivo* development of 2-cell stage embryos grade B and D reduced in comparison with the control group. The results of of studies by Choi *et al*.[28] as well as Lastra *et al*. [29] showed that Zn supplementation appears to have a positive effect on mouse embryo number and viability.

The positive effects of Zn supplementation on *in vivo* early embryonic development and the quality of embryos retrieved from aged mice after 24 hours of fertilization reported in this investigation may also be supported by previous studies which showed that the supplementation of Zn improved the quality of early embryonic development of the mouse [30].However, Zn promotes the *in vitro* maturation of oocytes and subsequently the embryonic development of sheep [31]. It has been reported that Zn enhances the development of the 2-cell stage embryo of pigs [32]. In this aspect, Zn enhances the size of the embryo's inner cell mass which may be linked with a greater pregnancy rate [33].

Mohsin *et al*. demonstrated that the count of neonates increased significantly in aged mice that received an extract of *Glycyrrhiza glabra* and it should be noted that Zn is one of the most essential basic elements in this plant [34]. On the other hand, inadequate maternal Zn consumption before and throughout pregnancy leads to effects on the placenta cardiovascular system which is likely to have an influence on in-utero programming of offspring development and growth [35].Additionally, Sanusi et al. reported that Zn deficiency has been related to adverse effects on maternal health and pregnancy outcomes [36]. Supplementary zinc up-regulated GSH synthesis and increased the quality of embryonic development [32]. GSH protect the embryo against ROS activity and decreased DNA damage significantly [37].

5. Conclusion

Consequently, the present study concluded that Zn supplementation for aged mice is essential for reproductive performance and embryonic development leading to physiologically normal litter size in mice. This finding can be used for other mammals to treat infertility and improve pregnancy outcomes.

6. Conflict of Interest

The authors declare that they have no conflict of interest.

References

[1] R. Fritz and S. Jindal, "Reproductive aging and elective fertility preservation" *Journal of Ovarian Research*, vol. 11, no. 66, pp.1-8, 2018.

[2] T. A. Ahmed, S.M. Ahmed, Z. El-Gammal, S. Shouman, A. Ahmed, R. Mansour and N. El-Badri, "Oocyte Aging: The Role of Cellular and Environmental Factors and Impact on Female Fertility, "*Advances in Experimental Medicine and Biology*, vol.8, pp.109-123,2020.

[3] K. R. Dunning, D. L. Russell, and R. L. Robker, "Lipids and oocyte developmental competence: The role of fatty acids and β-oxidation" *Reproduction*, vol. 148, no.1, pp.15–27, 2014.

[4] J. Li, L.Liu, J. Weng, T. Yin, J.L Yangand H.L.Feng, "Biological roles of L-carnitine in oocyte and early embryo development" *Mol Reprod Dev*, vol.88, pp. 673–685, 2021.

[5] A. Agarwal, A. Aponte-Mellado, B.J. Premkumar, A. Shaman, and S. Gupta, "The Effects of Oxidative Stress on Female Reproduction: A Review" *Reproductive Biology and Endocrinology*, vol. 10,pp. 10-49, 2012.

[6] S. Janati, M.A. Behmanesh, H. Najafzadehvarzi, Z. Akhundzade, and S.M. Poormoosavi, "Follicular fluid zinc level and oocyte maturity and embryo quality in women with polycystic ovary syndrome" *Int J Fertil Steril*. Vol.15 no. 3, pp. 197-201, 2021.

[7] A. Y. Alhijazi and D. H. Al-Bayati, "Influence of oral zinc sulphate supplementation on the development of the teeth and jaws of growing rat (Histological study)" *J Bagh College Dentistry*, vol. 23(special issue), pp. 53-58, 2011.

[8] A. Fallah, A. Mohammad-Hasani and A. Hosseinzadeh Colagar, "Zinc is an Essential Element for Male Fertility: A Review of Zn Roles in Men's Health, Germination, Sperm Quality, and Fertilization" *J Reprod Infertile*, vol, 19 no. 2, pp. 69-81,2018.

[9] E.A. Ali, H.K. Abdul Hussein and Y.H. Tahssen, "Estimation of Serum Copper, Manganese, Selenium, and Zinc in Hypothyroid Patients" *Journal of the Faculty of Medicine Baghdad*, vol. 50 no. 2, pp. 255–260, 2008

[10]D. D. Marreiro, K. J. Cruz, J. B. Morais, J. B. Beserra, J. S. Severo, and A. R. de Oliveira, "Zinc and Oxidative Stress: Current Mechanisms" *Antioxidants (Basel, Switzerland)*, vol, 6no.24, pp. 1-9, 2017.

[11]D. Bhowmik, C. Bhattacharjee, and S. A. Kumar, "potential medicinal importance of zinc in human health and chronic disease, " *Int. J. Pharm. Biomed. Sci*. vol.1, pp. 5–11, 2010.

[12]Z. Hussein, "Effects of the Zinc on Activity of Immune System in Male Albino Mice" *IBN AL-HAITHAM J. FOR PURE & APPL. SCI*. vol. 23, no. 1, 2010.

[13]S. S. Al-Dujaily, H. E. Al-Ahmed, J. A. Al-Ethawi, H. A. Al-Ebrahimi, "Effect of Fertility Blend® Administration on the Epididymal Sperm Function Parameters of Vasectomized Mice: Physiological and Genetical Study as Model for Obstructive Azoospermic Men" *Journal of Biotechnology Research Center*. Vol. 12, no.1, pp.132-136, 2018

[14]N. K. Hussain, S. S. Rzoqi, A.W. Numan, D. T. Ali, "A Comparative Study of Fructose, Zinc and Copper Levels in Seminal Plasma in Fertile and Infertile Men" *IRAQI J MED SCI*. vol.9, no.1, 48-54, 2011.

[15]L. Wang, R. Liang, G. Zhang, M. Ren, M. Long, J. Na, Z. Li, Bin Wang, L. Zhuang, Q. Lu," Serum zinc concentration and risk of adverse outcomes to in vitro fertilization and embryo transfer: A prospective cohort study in northern China,"*Science of The Total Environment*. Vol.7, 792, pp. 1-6,2021.

[16] S. R. Lisle, K. Anthony, M. A. Randall and F. J. Diaz, "Oocyte–cumulus cell interactions regulate free intracellular zinc in mouse oocytes, "*Reproduction.* vol,145, pp. 381–390,2013.

[17] T.B. Garner, J.M. Hester, A. Carothers, F.J. Diaz, "Role of zinc in female reproduction" *Biol Reprod*. Vol. 104, no. 5, pp.976-994, 2021

[18] J.Y. Duffy, C.M. Miller, G.L. Rutschilling, G. M. Ridder, M. S. Clegg, C. L. Keen, and G. P. Daston, "A decrease in intracellular zinc level precedes the detection of early indicators of apoptosis in HL-60 cells" *Apoptosis*. Vol. 6, pp.161–172, 2001

[19] L.A. Hanna, M. S. Clegg, T. Y. Momma, G. P. Daston, J. M. Rogers and C. L. Keen, "Zinc Influences the in Vitro Development of Peri-Implantation Mouse Embryos" *Birth Defects Research*. Vol. 67, pp. 414–420, 2003

[20] A. R. Mohsin,"Fertilization and Embryonic Development after Oral Administration of Lepidium meyenii (Maca) and Glycyrrhiza glabra Crude Aqueous Extract in Mice, "Ph.D. Dissertation, University of Baghdad, College of Science for Women. 2021.

[21]S.S. Al-Dujaily, "In vitro sperm activation and intra-barsal insemination in mice" PhD thesis, College of Veterinary Medicine, Baghdad University, 1996.

[22]B. Hogan, R. Beddington, Costantini F, E. Lacey, "Manipulating the mouse embryo: a laboratory manual, " 2nd ed NY: Cold Spring Harbor Laboratory, 1995.

[23] M.A. Khalili andM. Anvari, "The effect of *in vitro* culture on cleavage rates and morphology of the in vivo – developed embryos in mice" *Iran. J. Reprod. Med*. Vol. 5, pp. 17 – 22, 2007

[24] Statistical Analysis System, User's Guide. Statistical Version 28. SAS. Inst. Inc. Cary. N.C. USA, 2021

[25] V. Lodde, B. R Garcia, P.C. Dall'Acqua, C. Dieci, C. Robert, A. Bastien, M.A. Sirard, F. Franciosi, A.M. Luciano,"Zinc supports transcription and improves meiotic competence of growing bovine oocytes, "*Reproduction*. Vol.159, no. 6, pp. 679-691,2020.

[26] E.L. Que, F.E. Duncan, H.C. Lee, J.E. Hornick, S. Vogt, R.A. Fissore, T.V. O'Halloran and T. K. Woodruff, "Bovine eggs release zinc in response to parthenogenetic and sperm-induced egg activation" *Theriogenology*. Vol. 127, pp.41–48, 2019

[27] A.D. Mendoza, T.K. Woodruff, S.M. Wignall and T.V. O'Halloran, "Zinc availability during germline development impacts embryo viability in Caenorhabditis elegans, "*Comp Biochem Physiol C Toxicol Pharmacol*. Vol. 191, pp.194–202, 2017.

[28] Y.H. Choi, J.R. Gibbons, H.S. Canesin, andK. Hinrichs, "Effect of medium variations (zinc supplementation during oocyte maturation, perifertilization pH, and embryo culture protein source) on equine embryo development after intracytoplasmic sperm injection, "*Theriogenology*. Vol. 86, no.7, pp.1782–1788, 2016.

[29] M.D. Lastra, L. Saldívar, K. Martínez, N. Munguía, C. Márquez, A.E. Aguilar, "Zinc concentrations during mice gestation, " *Biol Trace Elem Res.* Vol. 105, pp. 205–214, 2005.

[30]S. Geravandi, M.Azadbakht, M. Pourmoradi, andF. Nowrouzi, "Zinc supplementation of vitrification medium improves in vitro maturation and fertilization of oocytes derived from vitrified-warmed mouse ovaries" *Cryobiology*. Vol. 74, pp. 31–35, 2017

[31] J. Su, Y. Yao, Y. Tang, H. Qin, R. Meng, C. Zhang, Y. Zhang, Y. Yang,P. Qiao, and J. Liu, " Zinc supplementation promotes oocyte maturation and subsequent embryonic development in sheep (preprint), " *Available at SSRN 4332376*.Scoups view. pp. 1-19, 2023

[32] Y. Jeon, J.D. Yoon, L. Cai, S.U. Hwang, E. Kim, Z. Zheng, E. Lee, D.Y. Kim, and S.H. Hyun, "Supplementation of zinc on oocyte in vitro maturation improves pre-implatation embryonic development in pigs, "*Theriogenology*. Vol. 82, no.6, pp. 866-874, 2014.

[33] L.K. Wooldridge, M.E. Nardi, and A.D. Ealy, "Zinc supplementation during in vitro embryo culture increases inner cell mass and total cell numbers in bovine blastocysts, "*Journal of Animal Science*. Vol. 97, no.12, pp. 4946–4950, 2019.

[34] A.R. Mohsin, S. S. Al-Dujaily, and M.K Haba, "Effect of novel licorice and maca combined plant crude aqueous extract on litter size and pups DNA normality in mice: experimental model for mammals, "*Plant Archives.* Vol. 20, no. 2, pp. 5015-5020, 2020.

[35] R.L. Wilson, S.Y. Leemaqz, Z. Goh, D. McAninch, T. Jankovic-Karasoulos, G.E. Leghi, J.A. Phillips, K. M. Colafella, C. Tran, S. O'Leary, S. Buckberry, S. Pederson, S.A. Robertson, T. Bianco-Miotto, C.T. Roberts, "Zinc is a critical regulator of placental morphogenesis and maternal hemodynamics during pregnancy in mice, " *Sci Rep*. vol. 7, no.1, 1-14 ,2017.

[36] K.O. Sanusi, K.G. Ibrahim, B. Abubakar, I. Malami, M.B. Bello, M.U. Imam, M.B. Abubakar, "Effect of maternal zinc deficiency on offspring health: The epigenetic impact, "*J Trace Elem Med Biol*. Vol. 65, pp. 1-9, 2021.

[37] S.J. Picco, J.M. Anchordoquy, D.G. de Matos, J.P. Anchordoquy, A. Seoane, G.A. Mattioli, A. L. Errecalde, and C.C. Furnus, "Effect of increasing zinc sulphate concentration during in vitro maturation of bovine oocytes, "*Theriogenology*. Vol. 74, no. 7, pp.1141–1148, 2010