BF-1541
[1-5]

RESEARCH ARTICLE   Indian Journal of Animal Research

Trace Elements in Pregnant and Non-pregnant Sheep and Goats and Their Relation to Steroid Hormones

Derar R. Derar[1,2], Ahmed Ali[1,2], Tariq I. Almundarij[1], Essam M. Abdel-Elmoniem[1,3], Tamim M. Alhassun[1], Moustafa Zeitoun[4]

10.18805/IJAR.BF-1541

ABSTRACT

Background: One of the major factors affecting the physiology of pregnancy and reproductive cycle is the trace elements intake and their levels in serum of brood ewes and does. The main objective of this investigation was to study the association between serum concentration of trace elements, ovarian findings, stage of pregnancy and steroid hormones in sheep and goats.

Methods: Total of 129 ewes (68 pregnant and 61 non-pregnant) and 54 does (32 pregnant and 22 non-pregnant) were examined clinically and ultrasonographically for pregnancy diagnosis and monitoring the ovarian findings. Pregnant animals were categorized according to the gestational stage into 1 to 2, >2 to 3, >3 to 4 and > 4 months. According to the ovarian findings, non-pregnant animals were categorized as having mature corpus luteum (CL), large follicle (> 5 mm) and corpus luteum (CL+F), growing follicles (GF, <5.mm) and mature follicle (MF, >5 mm). Animals were bled and serum was harvested and assayed for estrogen (E2), progesterone (P4), manganese (Mn), selenium (Se), iron (Fe) and zinc (Zn) levels.

Result: Mn (P=0.01), Fe (P=0.05) and Zn (P=0.001) were lower in pregnant compared with non-pregnant ewes. Fe was significantly higher (P=0.03) in non-pregnant compared with pregnant does at any gestational stage. Ewes with MF had higher Se (P=0.02) and Fe (P=0.001) compared with other ewes. Fe (P=0.001) and Zn (P=0.000) were higher in does having MF compared with other does. A positive association between E2 and serum Fe in non-pregnant ewes (P=0.03) and does (P=0.05) was found. It can be concluded that pregnancy associated with a decrease in serum trace elements in sheep and goats.

Key words: Goats, Ovarian structures Pregnancy, Sheep, Steroids, Trace elements.

INTRODUCTION

Trace elements are essential intermediaries in ovarian function and their involvement in both follicular development and hormone production is well documented (Yaman *et al*., 2007) in man and animals. Trace elements are found in different ovarian structures including mature follicles and corpora lutea even atretic follicles (Bhardwaj and Sharma, 2011). Iron for instance is indispensable for the ovulation process (Chavarro *et al*., 2006). Trace elements are necessary for maturation of the oocyte and maintaining the number of stored follicles at their normal range through their inevitable role in gonadotropins secretion (Chavarro *et al*., 2006; Singer *et al*., 2011). As a key player in the biosynthesis of cholesterol, they are irreplaceable for steroidogenesis and subsequent regulation of the folliculogenesis in female animals (Amin *et al*., 2016). Accordingly, animals receive trace elements-deficient diets are prone to failure of oogenesis, increased follicular atresia, inadequate steroidogenesis, ovulation failure and CL persistence (Gürdoðan *et al*., 2006; Vázquez-Armijo *et al*., 2011; Grace and Knowels, 2012; Ceko *et al*., 2016). Their association with the changes in the different ovarian events and the accompanied fluctuation on the level of steroid hormones during the reproductive cycle is crucial for the area of research interested in *in vivo* and *in vitro* manipulation of the mammalian oocyte. In this respect, further investigations are needed to fulfill this concept.

[1]Department of Veterinary Medicine, College of Agriculture and Veterinary Medicine, Qassim University, Saudi Arabia.
[2]Department of Theriogenology, Faculty of Veterinary Medicine, Assiut University, Assiut-71526, Egypt.
[3]Department of Soil, College of Agriculture and Veterinary Medicine, Qassim University, Saudi Arabia.
[4]Department of Animal Production, College of Agriculture and Veterinary Medicine, Qassim University, Saudi Arabia.

Corresponding Author: Derar R. Derar, Department of Veterinary Medicine, College of Agriculture and Veterinary Medicine, Qassim University, Saudi Arabia. Email: dr.mohammad@qu.edu.sa

How to cite this article: Derar, D.R., Ali, A., Almundarij, T.I., Abdel-Elmoniem, E.M., Alhassun, T.M. and Zeitoun, M. (2022). Trace Elements in Pregnant and Non-pregnant Sheep and Goats and Their Relation to Steroid Hormones. Indian Journal of Animal Research. DOI: 10.18805/IJAR.BF-1541.

Submitted: 16-05-2022 **Accepted:** 14-08-2022 **Online:** 07-09-2022

The normal requirements of trace elements increase greatly during pregnancy and adequate supply is of utmost importance before intended breeding to prepare the animal for the sequences of pregnancy and associated physiological changes during this period. Trace elements are involved in the growth, development and normal evolution of the pregnant uterus and its contents (Amin *et al*., 2016). Their roles in the enzymatic activity, efficiency of the immune system, the buildup of antioxidant capacity, integrity of the inner lining of the reproductive organs are non-negotiable (Uslu *et al*., 2017). Abortion, retarded growth, pre-term birth, stillbirth and birth of congenitally-deformed newborn are the result of trace elements-deficient diets. There were mixed responses to reports on the association between pregnancy and trace elements, regardless of the fact that the requirement for trace elements doubles or triples during pregnancy (Nawito *et al*., 2015; Spencer *et al*., 2015).

The present study aimed to study the association between the level of four major trace elements known for their deficiency in Qassim region; manganese (Mn), selenium (Se), iron (Fe) and zinc (Zn), ovarian findings, stage of pregnancy and level of steroids in non-pregnant and pregnant sheep and goats, respectively.

MATERIALS AND METHODS

Total of 129 ewes (68 pregnant and 61 non-pregnant) and 54 does (32 pregnant and 22 non-pregnant) in Qassim region, Saudi Arabia, were included in this study during the breeding season (autumn/winter, 2020). The study was carried out at the university teaching hospital, Qassim University. Animals aged 1-5 years, weighed 55-65 Kgs, average body condition score 3 (Kenyon *et al*., 2014) fed maintenance ration for breeding females (NRC, 1985 and 2007) and drinking water ad libitum.

The external genitalia of the studied animals were evaluated for their soundness. Transabdominal and transrectal ultrasonography using a 5 MHz transducer (Eickemeyer - Medizintechnik für Tierärzte KG, export office, Eltastraße 878532, Tuttlingen, Germany) for monitoring the reproductive tract of the studied ewes was carried out to diagnose pregnancy and to determine the ovarian findings in non-pregnant animals (because animals were not in the same stage of the cycle they were classified according to the ovarian findings). The built in software was used to determine the stage of pregnancy after freezing the screen at the best-fit sonogram for the placentomes or the fetal parts designed to determine the stage of pregnancy. The pregnant animals were categorized according to stage of pregnancy into 1 to 2, >2 to 3, >3 to 4 and > 4 months groups. Non-pregnant animals were, further, categorized according to the findings on their ovaries into those with corpus luteum (CL) vs. corpus luteum and large follicle [CL+F (>5 mm)] vs. growing follicles (GF, <5 mm) vs. mature follicle (MF, >5 mm) groups.

On admission, animals were venipunctured and bled into plain tubes at 8:00-10:00 a.m. and let at room temperature for 30 minutes. Centrifugation of the collected samples at 3000 xg was carried out for 15 minutes. The obtained serum was kept at -20°C until hormonal assay and estimation of trace elements.

Flame emission atomic absorption (Moffat *et al*., 1986) was used to estimate serum levels of Mn, Se, Fe and Zn after samples digested using HClO4-HNO3 mixture according to the technique described previously (Antoniou *et al*., 1995).

Levels of E2 and P4 in serum were determined by ELISA (kits brought from Prechek Bio., Inc., Young In city, Korea, Lot No: 119011502). The coefficients of variation of the intra-assays were 3.7 and 5.1%, inter-assays 6.4 and 6.8% for E2 and P4, respectively. Three pg/ml for E2 and 0.12 ng/ml for P4 were the sensitivity of the assays.

The data were expressed in means±SE. SPSS program, version 25 (SPSS Inc., Chicago, IL, USA, 2017) was used for statistical analysis. One way-ANOVA was used to test the difference between animals' groups. Relationships between trace elements and steroids were tested using the correlation coefficient. The level of significance was set at P<0.05.

RESULTS AND DISCUSSION

Trace elements were lower in pregnant compared with non-pregnant ewes and does. This decrease was significant for Mn (P=0.01), Fe (P=0.05) and Zn (P=0.001) in ewes (Table 1) and for Fe (P=0.03) in does (Table 2). Positive association between Fe and P4 (r=0.56; P=0.01), Fe and E2 (r=0.65; P=0.03) and Mn and P4 (r=0.61, P=0.04) in pregnant ewes. In the present study, Mn, Fe and Zn in sheep and Fe in does decreased significantly during pregnancy compared with open ewes and does. Though non-significant, Se decreased also in pregnant animals. These decreases were more obvious with the advancement of pregnancy. Similarly, it was found that the concentration of Se and other elements in the circulation falls considerably (12%) during normal pregnancy as gestation progresses in sheep (Ganie *et al*., 2014; Uslu *et al*, 2017). Another potential reason of this fall in serum trace elements in blood during pregnancy is these elements may be transported to the fetus by certain mediators, which are expressed in the placenta, thereby causing a fall in blood concentration of these elements during

Table 1: Serum manganese (Mn), Selenium (Se), iron (Fe), zinc (Zn), estrogen (E2) and progesterone (P4) during different gestational stages of pregnant (n= 68) compared with non-pregnant (n= 61) ewes.

Stage of pregnancy	n	Mn (μmol/L)	Se (μmol/L)	Fe (μmol/L)	Zn (μmol/L)	P4 (ng/ml)	E2 (pg/ml)
non	61	4.39 ± 1.10^{b}	0.69 ± 0.24^{a}	12.89 ± 3.14^{b}	6.75 ± 6.27^{b}	1.49 ± 0.72^{a}	110.34 ± 5.04^{b}
1-2	7	3.34 ± 0.34^{a}	0.50 ± 0.14^{a}	8.05 ± 1.89^{a}	3.87 ± 1.15^{a}	3.32 ± 0.26^{b}	79.65 ± 16.49^{a}
>2-3	30	2.47 ± 0.49^{a}	0.57 ± 0.05^{a}	8.37 ± 0.83^{a}	4.39 ± 0.35^{a}	3.25 ± 0.42^{b}	94.92 ± 8.39^{a}
>3-4	18	1.81 ± 0.42^{a}	0.77 ± 0.15^{a}	7.15 ± 0.96^{a}	4.65 ± 0.57^{a}	3.52 ± 0.53^{b}	89.42 ± 9.94^{a}
>4 months	13	1.87 ± 0.25^{a}	0.58 ± 0.09^{a}	8.22 ± 1.54^{a}	4.21 ± 0.78^{a}	3.88 ± 0.44^{b}	103.55 ± 8.27^{a}

Values are in means±standard error. Different superscript letters in the column are given for significantly different values (*P*<0.05).

pregnancy (Makhlouf *et al*., 2020; Derar *et al*., 2022). It has been speculated that progesterone mobilizes trace elements towards the reproductive organs especially the uterus particularly during pregnancy (Dalai *et al*., 2017) which may explain the decline in serum trace elements during pregnancy and the CL-dominant compared with the follicular-dominant statuses in non-pregnant animals in the present study. In addition, the reduction in trace elements during pregnancy may be the consequence of hemodilution from maternal plasma expansion, increased transport of these elements to the fetus and as a result of increased utilization for the synthesis of elements-dependent antioxidant proteins required to combat the increased oxidative demands of pregnancy (Spencer *et al*., 2015). Both Fe and Zn are involved in the epithelialization of the fetus. Zn is essential for DNA replication and protein synthesis of the fetal tissue not mentioning its role in the immune mechanism during the gestation period (Nawito *et al*., 2015; Dalai *et al*., 2017; Makhlouf *et al*., 2020).

Ewes with MF in their ovaries had higher Se (P=0.02) and Fe (P=0.001) compared with ewes having other structures in their ovaries (Table 3). Fe (P=0.001) and Zn (P=0.000) were higher in does having MF compared with does having other structures either GF or CL (Table 4). A positive association was found between E2 and serum Fe in non-pregnant ewes (r=0.7, P=0.03) and does (r=0.54, P=0.05).

Presence of mature follicles in the ovaries of ewes was associated with higher level of serum Se and Fe in the present study. Similarly, healthy follicles of different size categories from small to large size were associated with a rise in serum concentration of these elements in goats (Dalai *et al*., 2017). It was postulated that trace elements are essential for ovarian tissue build-up involving mitochondrial function, cell division, biosynthesis of estrogen and progesterone and combating free radicals (Gajda *et al*., 2008). These elements exert their action via induction of DNA synthesis, activation of adenyl kinase, phosphodiesterase, membrane–bound adenyl cyclase and lipid peroxidase in the process of follicle growth and maturation (Zheng *et al*., 2015).

Females with delayed sexual maturity, idiopathic infertility were found to have inadequate Se and aberrated Se-binding protein-1 (Edassery *et al*., 2010). Fe, Se and Zn were consistently confined to the granulosa cell layer of different classes of the ovarian follicles including small,

Table 2: Serum manganese (Mn), selenium (Se), iron (Fe), zinc (Zn), estrogen (E2) and progesterone (P4) during different gestational stages of pregnant (n= 32) compared with non-pregnant (n= 22) does.

Stage of pregnancy	n	Mn (µmol/L)	Se (µmol/L)	Fe (µmol/L)	Zn (µmol/L)	P4 (ng/ml)	E2 (pg/ml)
non	22	2.94±0.48[a]	0.62±0.13[a]	18.74±17.23[b]	5.87±1.02[a]	0.88±0.18[a]	112.64±10.30[a]
1-2	6	2.44±0.89[a]	0.49±0.03[a]	14.11±1.98[a]	5.52±1.10[a]	2.95±0.49[b]	76.73±12.11[b]
>2-3	8	2.28±1.13[a]	0.31±0.11[a]	11.08±1.33[a]	6.80±3.46[a]	3.50±1.44[b]	77.31±12.81[b]
>3-4	8	1.36±0.13[a]	0.20±0.07[a]	10.93±2.10[a]	4.13±0.72[a]	4.33±1.47[b]	91.47±16.10[b]
> 4 months	10	2.43±1.18[a]	0.65±0.15[a]	11.94±3.25[a]	4.39±0.53[a]	3.43±1.06[b]	96.48±9.33[b]

Values are in means±standard error. Different superscript letters in columns are given for significantly different values (*P*<0.05).

Table 3: Serum manganese (Mn), Selenium (Se), iron (Fe), zinc (Zn), estrogen (E2) and progesterone (P4) associated with different ovarian findings in non-pregnant (n = 61) ewes.

OVF	n	Mn (µmol/L)	Se (µmol/L)	Fe (µmol/L)	Zn (µmol/L)	P4 (ng/ml)	E2 (pg/ml)
CL	15	2.76±0.47[a]	0.35±0.06[a]	3.74±2.96[a]	3.79±0.94[a]	3.40±0.47[a]	116.01±6.72[a]
CL+F	14	2.80±0.38[a]	0.38±0.12[a]	3.34±1.23[a]	3.76±0.67[a]	3.19±0.71[a]	97.98±6.74[a]
GF	17	3.15±1.12[a]	0.63±0.10[a]	2.78±1.85[a]	5.98±1.48[a]	1.88±0.68[b]	105.07±10.01[a]
MF	15	2.16±1.57[a]	1.95±1.65[b]	6.71±1.38[b]	3.89±2.05[a]	1.90±0.59[a]	175.30±14.92[b]

Values are in means±standard error. Different superscript letters in the same column are given for significantly different values (*P*<0.05). CL: corpus luteum; F, follicle; GF, growing follicle (<5mm); MF, mature follicle (>5mm).

Table 4: Serum manganese (Mn), Selenium (Se), iron (Fe), zinc (Zn), estrogen (E2) and progesterone (P4) associated with different ovarian findings (OVF) in non-pregnant (n= 22) does.

OVF	n	Mn (µmol/L)	Se (µmol/L)	Fe (µmol/L)	Zn (µmol/L)	P4 (ng/ml)	E2 (pg/ml)
CL	5	1.72±0.06[a]	0.32±0.03[a]	11.03±3.09[a]	4.96±0.76[a]	4.82±0.33[b]	81.60±5.39[a]
CL + F	3	1.31±0.10[a]	0.81±0.08[a]	12.75±5.15[a]	5.17±0.55[a]	3.46±0.43[b]	104.10±4.38[a]
GF	7	2.63±0.35[a]	0.42±0.08[a]	12.91±0.82[a]	3.27±0.11[a]	1.04±0.60[a]	108.84±5.24[a]
MF	7	2.26±0.41[a]	0.94±0.03[a]	16.98±1.63[b]	6.04±0.74[b]	1.50±0.08[a]	138.09±5.92[b]

Values are in means±standard error. Different superscript letters in columns are given for significantly different values (*P*<0.05). CL, corpus luteum; F, follicle; GF, growing follicle (<5mm); MF, mature follicle (>5mm).

medium and large follicles (Ceko *et al*., 2015). It was reported that the elevated Se in healthy follicles is may be attributable to the upregulation of selenoproteins GPX1 in these follicles compared with atretic ones (Ceko *et al*., 2016). Based on these findings, there is strong evidence that these elements are decisive for different stages of follicular development including recruitment, selection, dominance and maturation (Edassery *et al*., 2010). It was found that Se and Zn are abundant in the follicular wall in both theca interna and the granulosa (Ceko *et al*., 2015). Trace elements especially Se and Zn are involved in cholesterol synthesis (Zheng *et al*., 2015). Cholesterol is the precursor for synthesizing estrogen and progesterone in human and animals. It has been reported that the relationship between trace elements and steroids is reciprocal and both act synergistically during the development and maturation of the Graffian follicle (Kumar *et al*., 2011).

The above concepts are supported by the positive association between steroids and trace element in the present study. The higher concentrations of Se and Fe in large follicles compared with other ovarian structures were reported before (Kor *et al*., 2013). As a result, the developing follicle shunt experiences increased hemodynamic pulses due to increasing levels of estrogen and progesterone (Abd Ellah *et al*., 2010). It was reported previously that serum trace elements levels increased significantly in response to higher estrogen levels, especially during the latter stages of the pre-ovulatory follicles, but then fell just before ovulation (Antunovic *et al*., 2002). It has been found that a positive interrelationship between serum levels of trace elements and the level of estrogen (Kumar *et al*., 2011). A rise in these elements was associated with extrinsic estrogen administration (Dalai *et al*., 2017). Researchers have found changes in plasma minerals associated with changes in plasma estrogen during the estrous cycle, following ovariectomy and during the administration of exogenous estrogen (Zheng *et al*., 2015). Despite the marked rise in serum trace elements during the follicular phase of the estrus cycle follicular contents of the trace elements decreased (Makhlouf *et al*., 2020). By synthesizing protein and metalloenzymes, the ovary synthesizes cations for ovarian development, as evidenced by a decrease in follicular fluid trace elements (Kor *et al*., 2013).

CONCLUSION

It can be concluded that pregnancy associates with a decrease in serum trace elements in sheep and goats. Accordingly, it is recommended that these elements should be supplemented to the animals before the intended breeding time. The positive association between trace elements and steroidogenic outcome of the mature follicles in sheep and goats should be considered when future application of reproductive biotechnology in these species.

Declaration of interest

None.

Compliance with ethical standards

The study was conducted in accordance with the Declaration of Helsinki. The animal Care and Welfare Committee for Qassim University, Saudi Arabia, approved this study (No. 321143).

Funding

None.

Contributions of the authors

Derar Refaat and Ahmed Ali have laid the concept of the research, collect samples, examined animals and wrote the manuscript; Tariq Almundarij revised the manuscript; Essam Moneim was responsible for assaying the trace elements, Tamim Hassoun helped in collecting data and clinical examination; M Zeitoun assayed steroid hormones.

REFERENCES

Abd Ellah, M.R., Hussein, H.A., Derar, D.R. (2010). Ovarian follicular fluid constituents in relation to stage of estrus cycle and size of the follicle in buffalo. Vet. World. 3(6): 263-267.

Amin, B.Y., Dar, R.R., Ali, A., Malla, J.A., Shubeena, S., (2016). Role of Micro-nutrients in Bovine Reproduction. Theriogenology Insights. 6(1): 57-65.

Antoniou, V., Zantopoulos, N., Tsoukali-Papadopoulou, H. (1995). Selected heavy metal concentrations in goat liver and kidney. Vet. Hum. Toxicol. 37(1): 20-2.

Antunovic, Z., Sencic, D., Speranda, M. (2002). Influence of the season and the reproductive status of ewes on blood parameters. Small Rum. Res. 45: 39-44.

Bhardwaj, J.K., Sharma, R.K. (2011). Changes in trace elements during follicular atresia in goat (*Capra hircus*) ovary. Biol. Trace Elem. Res. 140(3): 291-8.

Ceko, M.J., Hummitzsch, K., Bonner, W.M., Aitken, J.B., Spiers, K.M., Rodgers, R.J., Harris, H.H. (2015). Localization of the trace elements iron, zinc and selenium in relation to anatomical structures in bovine ovaries by X-Ray fluorescence Imaging. Microsc. Microanal. 21(3): 695-705.

Ceko, M.J., O'Leary, S., Harris, H.H., Hummitzsch, K., Rodgers, R.J. (2016). Trace Elements in Ovaries: Measurement and Physiology, Biol. Reprod. 94(4): 1-14.

Chavarro, J.E., Rich-Edwards, J.W., Rosner, B.A., Willett, W.C. (2006). Iron intake and risk of ovulatory infertility. Obstet. Gynecol. 108(5): 1145-52.

Dalai, N., Das, K., Das, P.K., Ghosh, P.R., Shekhar, S., Pandiyan, G.D.V. (2017). Ovarian biometry with role and distribution of trace elements in ovarian follicular fluids of goat: An overview. Ind. J. Anim. Health. 56(2): 145-156.

Derar, D.R., Ali, A., Almundarij, T.I., Elmoniem, E.M.A. and Alhassun, T.M. (2022). Factors affecting serum trace elements of breeding ewes in central Saudi Arabia. Indian Journal of Animal Research. DOI: 10.18805/IJAR.BF-1475.

Edassery, S.L., Shatavi, S.V., Kunkel, J.P., Hauer, C., Brucker, C., Penumatsa, K., Yu, Y., Dias, J.A., Luborsky, J.L. (2010). Autoantigens in ovarian autoimmunity associated with unexplained infertility and premature ovarian failure. Fertil. Steril. 94(7): 2636-41.

Gajda, M., Banaś, K., Banaś, A., Jawień, J., Mateuszuk, Ł., Chłopicki, S., Kwiatek, W.M., Cichocki, T., Falkenberg, G. (2008). Distribution of selected elements in atherosclerotic plaques of apoE/LDLR-double knockout mice assessed by synchrotron radiation-induced micro-XRF spectrometry. X-Ray Spectrom. 37: 495-502.

Ganie, A.A., Baghel, R.P.S., Mudgal, V., Aarif, O., Sheikh, G.G. (2014). Effect of Selenium supplementation on reproductive performance and hormonal profile in buffalo heifers. Indian Journal of Animal Research. 48(1): 27-30.

Grace, N.D., Knowles, S.O. (2012). Trace element supplementation of livestock in New Zealand: Meeting the challenges of free-range grazing systems. Vet. Med. Inl. Article ID 639472. http://dx.doi.org/10.1155/2012/639472.

Gürdoğan, F., Yildiz, A., Balikci, E. (2006). Investigation of serum Cu, Zn, Fe and Se concentrations during pregnancy (60, 100 and 150 days) and after parturition (45 days) in single and twin pregnant sheep. Tur. J. Vet. Anim. Sci. 30: 61-64.

Kenyon, P.R., Maloney, S.K., Blache, D. (2014). Review of sheep body condition score in relation to production characteristics NZ. J. Agri. Res. 57(1): 38-64. http://dx.doi.org/10.1080/00288233.2013.857698.

Kor, N., Khanghah, K., Veisi, A. (2013). Follicular fluid concentrations of biochemical metabolites and trace minerals in relation to ovarian follicle size in dairy cows. Ann. Res. Rev. Biol. 3(4): 397-404.

Kumar, S., Kumar, P.A., AbdulRazzaque, W.A., Dwivedi, D.K. (2011). Importance of micro minerals in reproductive performance of livestock. Vet. World. 4(5): 230-233.

Makhlouf, A., Titaouine, M., Mohamdi, H., Yakoub, F. (2020). Effect of different altitude on reproductive performances and mineral assessment in Ouled Djellal ewes during the mating period. Trop. Anim. Health Prod. 52: 3275-3283.

Moffat, A.C., Jackson, J.V., Moss, M.S., Widdop, B. (1986). Metals and Anions. In: Clarke,s Isolation and Identification of Drugs. 2nd Ed., The Pharmaceutical Press, London.

National Research Council, (1985). Nutrient Requirements of Sheep. Sixth edition. National Academy Press. Washington, DC, USA:

National Research Council (2007). Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids and New World Camelids. The National Academies Press. Washington, DC: https://doi.org/10.17226/11654.

Nawito, M.F., Mahmoud, K.G.M., Kandiel, M.M.M., Ahmed, Y.F., Sosa, A.S.A. (2015). Effect of reproductive status on body condition score, progesterone concentration and trace minerals in sheep and goats reared in South Sinai, Egypt. Afr. J. Biotech. 14(43): 3001-3005.

Singer, S.T., Vichinsky, E.P., Gildengorin, G., van Disseldorp, J., Rosen, M., Cedars, M.I. (2011). Reproductive capacity in iron overloaded women with thalassemia major. Blood. 118(10): 2878-81.

Spencer, B.H., Vanderlelie, J.J., Perkins, A.V. (2015). Essentiality of trace element micronutrition in human pregnancy: A systematic review. J. Preg. Child Health 2: 157. doi: 10.4172/2376-127X.1000157.

Uslu, B.A., Mis, L., Gulyuz, F., Comba, B., Ucar, O., Tasal, I., Comba, A., Kosal, V., Sendag, S., Wehrend, A. (2017). Is there a relationship between serum minerals (Ca, Mg) and trace elements (Cu, Fe, Mn, Zn) at mating on pregnancy rates in fat-tailed Morkaraman sheep? Indian J. Anim. Res. 51(2): 256-262.

Vázquez-Armijo, J.F., Rojo, R., Salem, A.Z.M., López, D., Tinoco, J.L., González, A., Pescador, N., DomÃ-nguez-Vara, I.A. (2011). Trace elements in sheep and goats reproduction: A review. Trop. Subtrop. Agroecosys. 14: 1-13.

Yaman, M., Kaya, G., Yekeler, H. (2007). Distribution of trace metal concentrations in paired cancerous and non-cancerous human stomach tissues. World J. Gastroenterol. 13: 612-8.

Zheng, G., Wang, L., Guo, Z., Sun, L., Wang, L., Wang, C., Zuo, Z., Qiu, H. (2015). Association of serum heavy metals and trace element concentrations with reproductive hormone levels and polycystic ovary syndrome in a chinese population. Biol. Trace Elem. Res. 67(1): 1-10.