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A trial to pinpoint some causes of infertility in ewes from the New Valley government

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Abstract

Many factors were found to affect ovarian activity negatively in farm animals. Last year's researches focused on several natural compounds (heavy metals and phytoestrogens) that may interfere with the major functions of the endocrine system and can affect female fertility .Therefore ,they were termed endocrine disruptors (EDCs) . So , the objective of the present study was to pinpoint some causes of infertility in ewes from the El-Dakhla city (New Valley –Egypt) as some EDCs (iron, lead and cadmium) may present in well-water and berseem fodder as well as some trace elements and minerals that affect biosynthesis or activity of FSH ,LH, estradiol ,progesterone ,testosterone ,T3 ,T4 and TSH .A total of thirty 3-6 years old Balady ewes (30-40 kg) were studied .The control ewes (G1 ,n =10) were fed according to their physiological status and were of normal fertility. Twenty infertile ewes were from two different farms(ten for each ,G2& G3) and had been grazed on a highly estrogenic pasture of Berseem clover (*Trifolium alexandrinum* ,Al -Abbd) rich in phytoestrogen and drink well-water directly from the same well without any treatment. Well-water, berseem and serum samples were analyzed for their heavy metals content. Serum samples were analyzed for minerals, and hormonal levels. The obtained results revealed that well-water and berseem fodder had iron levels higher than the permissible limits of WHO and Egyptian ministry of health .Meanwhile, Pb and Cd levels were within the permissible limits .Calcium level of well-water was within the permissible limit of WHO. Serum analysis recorded drastic increase in Fe levels but the Pb and Cd levels were not detected . Insignificant increase was recorded in Ca levels in both the infertile groups. Zn concentrations were significantly decreased in G2& G3 ,Cu levels were significantly decreased in G2 and Pi levels decreased significantly in G3 only. Serum hormonal analysis showed significant decrease in LH levels in G3 with significant decrease in FSH levels in G2&G3 .Moreover , estradiol and progesterone concentrations were significantly increased in both the infertile groups. Meanwhile , testosterone levels significantly increased& decreased in G2&G3 , respectively. Significant decrease in T3 levels in both the infertile groups .Also, significant increase was recorded in T4 &TSH levels in G2&G3 in comparing with the control group. In conclusion , this study indicated that heavy metals pollution of well-water and berseem fodder with iron as well as serum zinc ,copper and phosphorus deficiency affect fertility of ewes at El- Dakhla city farms . Moreover , grassing on berseem clover (*Trifolium alexandrinum* , Al -Abbd) may be the cause of reduced ovulation rate owing to high content of phytoestrogens. So, iron and phytoestrogen act as endocrine disrupting compounds which affect gonadotrophic hormones (LH&FSH), steroidogenesis and thyroid hormones production that affect ewes fertility.

Introduction

Small ruminant production represents the principal economic output, contributing a large share of the income of farmers. Therefore, goats and sheep play a significant role in livelihoods of the rural populace and urban dwellers in most developing countries. They are multi – functional animals and play a significant role in the economy and nutrition. Apart from serving as a vital protein source, they also provide income for meeting urgent household needs (**Oluwatomi, 2010)**.

Ovarian activity is a very important function for the furtherance of a species. Many factors were formed to affect ovarian activity negatively in farm animals and are related to nutrition, climate, housing, environmental pollution, physiology, health status and other miscellaneous factors (**Ahmed ,2007**) . Disturbed ovarian activity is the most prominent cause of reproductive failure and economic losses. It occurs in four clinical forms: silent heat, anestrus, cystic ovarian disease (COD) and premature luteolysis or persistence of CL (**Ahmed , 2006**) .

Last years researches focused on several natural and synthetic compounds that may interfere with the major functions of the endocrine system and were termed endocrine disruptors (EDCs) which are defined as chemical substances with either agonist or antagonist endocrine effects in human and animals. These effects may be achieved by interference with the biosynthesis or activity of several endogenous hormones(**Georgescu et al.,2006**) .

Recently, it was demonstrated that heavy metals such as cadmium (Cd), iron (Fe), mercury (Hg), nickel (Ni), lead (Pb) and zinc (Zn) may exhibit endocrine-disrupting activity in animals. Emerging evidence of the intimate mechanisms of action of these heavy metals is accumulating. It was revealed that the Zn atom from the Zn fingers of the estrogen receptor can be replaced by several heavy metal molecules as copper, cobalt, Ni and Cd. By replacing the Zn atom with Ni or copper, binding of the estrogen receptor to the DNA hormone responsive elements in the cell nucleus is prevented. In both males and females, low-level exposure to Cd interferes with the biological effects of steroid hormones in reproductive organs. Arsen has the property to bind to the glucocorticoid receptor thus disturbing glucocorticoids biological effects. With regard to Fe, this may induce alterations in male and female fertility, may affect the function of the hypothalamo-pituitary-thyroid axis or the hypothalamo-pituitary-adrenal axis, and disrupts biosynthesis of steroid hormones (**Georgescu et al.,2011**) .

Natural chemicals found in human and animal food (e.g., phytoestrogens, including genistein and coumestrol) can also act as endocrine disruptors . They often have a phenolic moiety that is thought to mimic natural steroid hormones and enable EDCs to interact with steroid hormone receptors as analogs or antagonists (**Diamanti-Kandarakis et al.,2008**) .So , Cows and ewes fed estrogenic forage may suffer from impaired ovarian function, often accompanied by reduced conception rates and increased embryonic loss . The infertility is temporary, normally resolving within 1 month after removal from the estrogenic feed. However, ewes exposed to estrogen for prolonged periods may suffer from a second form of infertility that is permanent, caused by developmental actions of estrogen during adult life. The cervix becomes defeminized and loses its ability to store spermatozoa, so conception rates are reduced(**Adams, 1995).**

Also, minerals and trace elements deficiency affect the appetite, early postnatal growth, immunocompetency and reproductive performance on a herd basis, especially when mineralized salts is not included in the ration . In sheep, it was found that copper deficiency due to high molybdenum and sulfur leads to stopped signs of estrus in old ewes and anestrus during the breeding season. Also, deficiency of phosphorus, iodine, copper and manganese interfered with ovarian activity and disturbed cyclicity **(Braun ,1997)** . In Egypt, studies on animals, plants and soil trace elements revealed that Egyptian livestock are involved in more than half of the nutritional deficiency diseases caused by trace elements. The most important deficient elements are copper and zinc .Zinc deficiency impairs synthesis/ secretion of FSH and LH **(Smith and Akinbamijo ,2000) .**So **,** understanding the adverse conditions affecting ovarian activity is an important step towards the development of management practices that positively influence the reproductive efficiency of farm animals . The objective of the present study was to pinpoint some causes of infertility in ewes from the El-Dakhla city (New Valley –Egypt) as some EDCs (iron , lead and cadmium)that may present in well water and berseem fodder , some minerals and trace elements that affect biosynthesis or activity of FSH and LH, estradiol, progesterone, testosterone, T3 ,T4 and TSH .

Materials and Methods

Thirty 3-6 years old Balady ewes (30-40 kg) from El –Dakhla City (New Valley –Egypt) were studied . The control ewes (G1, n =10) were fed according to their physiological status (NRC, 1985) and were of normal fertility. Clean treated water that is used for human being and mineral blocks were available for them all the time . Twenty infertile apparently healthy ewes were from two different farms(ten for each ,G2& G3) and had been grazed on a highly estrogenic pasture of Berseem clover

(*Trifolium alexandrinum* , Al- Abbd) rich in phytoestrogen and drink well-water directly from the same well without any treatment .

This trail was conducted to pinpoint some causes of infertility in these ewes . So, some heavy metals which act as endocrine disrupting compounds (iron ,lead and cadmium) were determined in well- water and berseem fodder to evaluate their effects on some reproductive and thyroid hormonal levels . Also, some metals ,trace elements and minerals were measured (iron ,lead ,cadmium , copper , zinc ,calcium and phosphorus) in serum of the control and infertile ewes .

Sampling:

1- Water and vegetation samples :

Five samples of the drinking well water were collected from the sheep farms, acidified with nitric acid (obtained from Merck) and stored at 4 ºC till analysis . Also, five samples of Berseem clover (Al -Abbd) were finely chopped, washed very well and rinsed in distilled water 3 times for 5 min each . The samples were then
placed in clean acid washed glass bottles (the washing was by soaking for 24h in the bath containing 10 % HNO_3 solution and for 24h in ultra-pure water) and stored at -20 ºC till subsequent digestion by using the method of **Kacar (1972)** and analysis .

A -Analysis of Water and vegetation samples for the heavy metals :

The prepared extracts were filtrated with ash less filter papers and transferred to 10 ml digestion tubes to apply analysis by atomic absorption spectrophotometer (Sens AA DUAL ,GBC Scientific Equipment) at Toxical unit , Biochemistry Department , Animal Health Research Institute , Docked . The absorbance and concentration were recorded directly on digital scale of the spectrophotometer and the levels of iron, lead and cadmium can be estimated according to **APHA (1998)** .

B- Analysis of calcium in water :

Calcium concentration in five well - water samples was analyzed in the laboratory within 24-72 hours. The estimation was carried out according to **Glinder and King (1972) .**

2 -Blood samples and adopted methods : -

Blood samples (5 ml) were randomly collected at morning, from each group, by jugular vein puncture during the breeding season (December ,2014) . Blood collected before feeding and drinking and centrifuged at 4000 rpm for 15 minutes. Blood serum samples were carefully separated and stored at -20 ºC until analysis .

A-Heavy metals , trace elements and mineral determination:

Serum iron (Fe) , lead (Pb),cadmium (Cd) ,zinc (Zn) and copper (Cu), levels were estimated by using atomic absorption spectrophotometer . Concentrations of calcium (Ca) and inorganic phosphorus (Pi) were detected in serum samples using colorimetric method (Bio- diagnostic kit) according to **Fraser et al.(1987)** .

B-Hormonal determination

Quantitative determination of serum estradiol and testosterone were carried out using DBC (Diagnostics Biochem Canada Inc.)enzyme immune assay kits, catalog No. CAN-E-430 and CAN-TE-250, respectively. Progesterone, Triiodothyronine (T3), thyroxin (T4) and thyroid stimulating hormone (TSH) were determined by using ELISA kit (Immunospec corporation , USA, catalog No .E29-197 , E29-229 , E29-230 and E29 -227, respectively). The assay based on a solid phase enzyme- linked immunosorbent assay with sensitivity 10pg/ml , 0.022 ng/ml , 0.2ng/ml , 0.25 ng/ml ,0.5 ug/dl and 0.2 uIU/ml for estradiol , testosterone , progesterone , T3 ,T4 and TSH, respectively.

Quantitative determinations of serum LH and FSH were carried out using sheep ELISA kits (Abnova , catalog No.KA2322 and KA2320 , respectively) .The minimal detectable concentration of ovine LH and FSH by this assay is estimated to be about 0.5 ng/ml .

Statistical analysis:

Data obtained were statistically analyzed according to **Snedecor and Cochran (1974)** using student's "t" test . Also, data analyzed using analysis of variance (ANOVA) using F- test according to **SPSS-18 (2009).**

Results and discussion

In many developing countries over the years, ground water remains one of the dependable sources of usable water in fast growing towns and villages where the supply of portable water is not consistent **(WHO,2002)** . Groundwater pollution is an undesirable change in groundwater quality resulting from human activities which can alter the natural composition of it through the disposal or dissemination of chemicals and microbial matter at the land surface and into soils, or through injection of wastes directly down the soil profile **(Muhammad ,2012)** . Iron is one of the most abundant metal in the earth crust and is essential for plant and human being, but excess iron in drinking water is toxic and produces inky taste and muddy smelling **(Sharma and Kaur,1997).**

The reported heavy metal concentration levels in well-water from sheep farms at El Dakhla City (New Valley ,Egypt) revealed that iron level (table ,1)was significantly ($P \leq 0.0001$) higher than that stated by **WHO (2002) and EMH (Egyptian Ministry of Health ,1995) .**

The color of the collected water samples confirmed this result because of the noticed rusty color in the samples .This is due to high Fe concentration as , iron salts are unstable and precipitated as insoluble iron hydroxide, which settles in as a rust-coloured silt. Anaerobic ground waters may contain iron at concentrations of up to several milligrams per liter without discoloration or turbidity in the water when directly pumped from a well, although turbidity and color may develop in piped systems at iron levels above 0.05–0.1 mg/litre (**Department of National Health and Welfare,1990).** Also, in well-water, iron concentrations below 0.3 mg/litre were characterized as unnoticeable, whereas levels of 0.3–3 mg/litre were found acceptable (**WHO,1996**) .Moreover ,our results were confirmed by the results of **Sharkawy and Snosy (2014)** who recorded high level of Fe than the permissible limit in the camel milk samples at El -Dakhla City .In another study ,**Sanjoy and Rakesh (2013)**assessed the concentration of Fe level in the ground water of Imphal Valley, India and found that it was above the highest desirable limit of 0.1 ppm . The concentration of Cu, Cd, Fe, Cr, Mn, Pb and Zn in ground water at Dhanbad, Bihar was studied by **Prasad and Jaiprakas (1999)** were found to be below the permissible levels although concentration of Fe and Mn was found above the permissible limits at a few stations . In anaerobic ground water where iron is in the form of iron (II), concentrations will usually be 0.5–10 mg/litre, but concentrations up to 50 mg/litre can sometimes be found. Concentrations of iron in drinking-water are normally less than 0.3 mg/litre but may be higher in countries where various iron salts are used as coagulating agents in water-treatment plants and where cast iron, steel, and galvanized iron pipes are used for water distribution **(WHO,1996)** .Meanwhile **, Scharawae et al. (2014)** reported that Fe level in well-water at sheep and goat farms in El –Menofia Governorate was within the permissible limit but lead level was above WHO limit .

Data in table(1) showed that lead and cadmium levels in well-water were within the permissible limits of **WHO (2003**) and **EMH (1995) .**These results were in agreement with those of **Sharkawy and Snosy (2014)** as they found that Pb level was within the permissible limit but Cd level was above **WHO(2003)** limit . However ,**Sanjoy and Rakesh (2013**) found that Pb and Cd levels in the ground water were greater than MPL of **WHO(1990) .**

The estimated values of calcium in well- water (table,1) were significantly($P \leq 0.001$) below the desirable limit specified by **WHO (2002)** . Several ecologists had regarded 1–15 ppm $CaCO_3$ content as nutrient poor, 16–60 ppm is moderate and above 60 ppm as nutrient rich. The present study reveals that the water samples are moderately nutrient rich **. Sanjoy and Rakesh (2013**) compared the calcium concentration in surface and ground water and found that ground water is slightly greater than surface water and moderately rich .In contrast ,**Ishaya and Abaje (2009)** detected the concentration of calcium from collected samples of bore well and showed varied results, with high concentration above 75mg/l **WHO(2002)** acceptable limit around the unplanned area of the town.

Heavy metals from industrial waste contaminate drinking water, soil, fodder and food. The toxic heavy metals like Cd, Pb and Fe affect biological functions, hormone system and growth . In general, the hazardous effects of these toxic elements depend upon the dietary concentration of the element, absorption of it by the digestive system and homeostatic control of the body for the element **(Rajaganapathy et al.,2011) .**

As shown in table (2) the concentration of heavy metals in berseem fodder (*Trifolium alexandrinum* , Al abbd) cultivated in El -Dakhla (New Valley ,Egypt) recorded high significant ($P \leq 0.0001$) levels of iron above the maximum permissible limit of **WHO(1999)** . Meanwhile ,the levels of lead and cadmium were within the maximum permissible limit of European Union (EU) food standards (**EC ,2006) . Rajaganapathy et al. (2011)** reported that the Cu and Fe content of soil and water significantly affected their respective concentrations in green berseem, sorghum stover and wheat straw. **Gowda et al. (2003)** found that the Pb (2.40-145 ppm), Cd (0.50-10 ppm) and Iron (3.38-11.6 ppm) content in the vegetation in an industrial area was higher as compared to normal areas .The current results were confirmed by the results of **Sharkawy and Snosy (2014**) who recorded high level of Fe with low level of Pb than the maximum permissible limit of Egyptian Standers in camel milk at El Dakhla region . They discussed that levels due to the feedstuff and well- water given to the animals were polluted with iron . Moreover ,**Yahaya et al. (2010)** assessed that the increase in industrial and agricultural processes has resulted in increased concentration of metals in the air, water and soil. These metals are taken in by plants and consequently accumulate in their tissues. In addition , The mean iron levels in vegetables samples was above the maximum permissible 0.3 ppm **(WHO, 1999)**, this may be due to a number of factors that influence the concentration of mineral elements on and within plants, these factors include climate , atmospheric deposition , nature of soil on which the plant is grown , irrigation with waste water **(Sa'eed and Abdullahi ,2012).**

The reproduction of small ruminants like goats and sheep managed under extensive range grazing conditions can be affected by nutrients availability and especially by the mineral content of the forages resources on the rangeland. It has been particularly demonstrated that trace elements can have equally, beneficial or detrimental effects, depending on its balance, on reproductive functions in small ruminants **(Vázquez-Armijo et al., 2011)** . Some heavy metals have bio-importance as trace elements and are essential for all living organisms such as Mn, Fe, Cu and Zn but the biotoxic effects of many of them in human and animal biochemistry are of great concern and constitute major contaminants such as Pb and Cd **(Duruibe et al., 2007) .**

The current study revealed that serum iron levels (table ,3) were increased significantly ($P \leq 0.05$) in both infertile groups of balady ewes at El- Dakhla City farms comparing with the fertile group .This may be due to drinking well-water and grassing on berseem fodder which have high level of iron .**Yahaya et al. (2010)** mentioned that the increase in heavy metals in the air, water and soil are taken in by plants and consequently accumulate in their tissues. Animals that graze on such contaminated plants and drink from polluted water accumulate such metals in their tissues and blood .

Researches on mineral concentration and interrelationship in soil, forage and blood serum of sheep and goats in Mexico have shown results that there are imbalances of minerals, with excesses of P and iron (Fe) in the sheep, with Cu deficiency associated wirh deficiencies of Cu and excesses of Fe in the soil and in forages **(Domínguez-Vara and Huerta- Bravo, 2008)** .Also **,Raj et al. (2006)** denoted high amount of heavy metals in serum samples of animals consuming fodder grown in land irrigated by polluted water with a mean concentration of Cd, Pb and Fe of 0.022, 0.385 and 24.16 ppm, respectively. Meanwhile, the levels of lead and cadmium were not detected in the serum of all groups .This result confirm our results of lead and cadmium in well-water and berseem fodder . The current results disagree with those of **Sharkawy and Snosy (2014)** who recorded high level of Cd in camel milk as a result of the contamination of this region (El- Dakhla city) with Cd but the Pb level was within the permissible limit.

Zinc is an essential element required by ruminants for a number of biochemical functions .Zn deficiency can affect growth, reproduction, immune system and gene expression in ruminants by influencing the enzyme activity or by its effect on mitogenic hormones, signal transduction, gene transcription and RNA synthesis. Adequate concentration of Zn in the serum and in diet, is vital for uterine involution, tissue repair, after parturition, and particularly, the return to estrus **(MacDonald, 2000) .**

Concerning the serum Zn levels ,the present study showed significant ($P \leq 0.05$) decrease in the infertile groups (table ,3)when compared with the control group .The deficiency of Zn in these animals may be due to grassing on berseem which is deficient in Zn and Cu as reported by **Kumar et al. (2009)** .Moreover ,**Ramirez (2009)** found that mineral concentrations of tree leaves and grasses consumed by goats in the southern Mexico State led to poor levels of minerals reported for Cu and Zn in serum of goats showed a marginal deficiency of Cu and Zn . **Kumar et al. (2011)** reported that Zn deficiencies have profound effect on reproductive cycle and pregnancy. Delayed puberty and lower conception rates, failure of implantation and reduction of litter size are also found in association with the zinc deficiency.

Low fertility associated with delayed or suppressed estrus and prolonged postpartum periods, as well as abortions and fetal losses, are reproductive disorders commonly found in Cu deficient animals, and animals with excess of Mo and/or S **(Vázquez-Armijo et al. 2011) .**

The results also showed that there was drastic Cu deficiency in G2 and insignificant decrease in G3 as compared to the control group (table ,3) .This deficiency may be due to the interaction between Cu and Mo which can result in a poor use of Cu .Mo interferes with the metabolism of Cu at the molecular level, forming chelates in the rumen which reduces its absorption, highly linked to the presence of sulfur (S) . In the rumen thiomolybdates are formed, by reactions between Mo and S . The thiomolybdates react with free Cu atoms, to form insoluble Cu complexes, thus, forming Cu-Mo-S complexes, affect Cu utilization and causes Cu deficiency **(Quiroz-Rocha and Bouda, 2001**). Also, **Hemken et al. (1998)**stated that although low Cu content of feedstuffs is a common cause of Cu inadequacy, reducing bioavailability of Cu in ruminants may occur when dietary sulphur, molybdenum, or iron are high . So , the high concentration of iron in water and berseem fodder was the cause of Cu deficiency in the current study.

As regards serum Ca levels , the present study revealed that there was insignificant increase in Ca level in both the investigated groups (table ,3) .This increase may be due to high estrogen levels recorded in these animals which cause increase of Ca retention (**Swenson ,1998)** .In addition ,**Yano et al. (1991)** reported that the increase in estrogen was in parallel with the increase of Ca absorption from the intestines . Moreover , the increase in Ca level may be due to some heavy metal pollution which causes disturbances in calcium/phosphorus homeostasis , which led to increased calcium level and significantly decreased the phosphorus level. This effect is due to the high bioavailability of the heavy metals and its long duration of action **(Rajaganapathy et al. ,2011)** .

A significant ($P\leq 0.05$) decrease in the inorganic phosphorus levels in G3 comparing with G2 and the control (table ,3) . This agrees with **Tag El –Din et al.(2005)** who recorded that the exposure of balady ewes to high levels of heavy metals in Shobra El –Khema decreased Pi concentration and attributed that to the anorexia and malabsorption of Pi from the intestine .The deficiency of Pi in sheep causes estrus suppression and poor conception rates **(Vázquez-Armijo et al. 2011)** .

In fact, endocrine disruption can take place at different physiological levels: A) altering (inhibiting or stimulating) the secretion of hormones; B) interfering with hormone-receptor interaction as it can act as agonist or antagonist by direct binding to the receptor or by interfering the transductional pathway of a hormone; C) modifying the metabolism of circulating hormones, by increasing or decreasing their excretion rate and or biotransformation in the liver, hepatopancreas or other organs **(Rodriguez et al., 2007) .**

Looking over table (4) , the obtained results revealed that there was significant($P\leq 0.05$) decrease in the level of serum LH in G3 as compared with G2 and the control group. Meanwhile, a significant decrease was recorded in FSH levels in both the investigated groups comparing with the control group .This decrease may be due to pollution of the drinking well-water and berseem fodder with iron .This explanation was confirmed by **Saputo (2011**) who saied that in hemochromatosis, excess iron can be built up in the hypothalamus or the pituitary, and sometimes in both locations. The iron damages cells in these areas resulting in LH and FSH production slows or stops, and the testes or ovaries stop functioning, a situation called hypogonadism .Also, **Ahmed (2007)** reported that chronic iron , copper and zinc intoxication in sheep from industrial emission caused the accumulation of them in the ovaries and uterus with disturbed ovarian activity and increased number of atretic follicles .The present results agree with **Boland (2003)** who found that Zn deficient animals have shown lower concentrations of FSH and LH .In addition , **Quiroz-Rocha and Bouda (2001)** induced a secondary Cu deficiency in ewes by means of Mo and S supplementation, this procedure suppressed estrous behavior, however, the females continued ovulating, based on this, the results suggested that by elevating Mo and S, production and /or expression of hormones, such as estrogens and luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were altered **.** Under nutrition increases the biosynthesis of neuropeptide Y (NPY) and endorphin and decreases biosynthesis of LH-releasing hormone-messenger ribonucleic acid (LH-RH-mRNA) in the hypothalamus. Such changes reduce the production and release of FSH or LH from the pituitary gland (**Ahmed ,2007). Ahmed and Ezzo (1998)** noticed that low levels of thyroid hormones were associated with a high level of thyroid stimulating hormone (TSH) and suppressed the synthesis, release, ratio and feedback mechanism of both FSH and LH and lead to cessation of ovarian activity .The results in this study go hand –to- hand with that of **Archibong and Abdelgadir (2000)** who stated that farm animals continuously fed on Berseem clover (*Trifolium alexandrinum*) or soybean showed reduced ovulation rate owing to high content of phytoestrogen and limitation of LH release. Also, reduction of conception rate frequently occurred in cattle grazing in pastures containing estrogenic plants through maintaining continuous uterine contractility . **Arispe et al.(2013)** demonstrated that selected phytoestrogens (coumestrol ,zearalenone and genistein),like estrogen act directly at pituitary loci to decrease basal secretion of FSH ,reduce total FSH production and enhance GnRH agonist with induced LH secretion in a manner that is dependent on the estrogen receptor .

As observed in the current study , there was a drastic significant ($P\leq 0.05$) increase in the levels of estradiol and progesterone (table ,4) in G2 and G3 comparing with G1 . Meanwhile ,a significant($P\leq 0.05$) increase and decrease in testosterone levels was recorded in G2 and G3 , respectively as compared with the control group . The increase in estradiol levels may be due to the disturbance in steroidogenesis due to the effect of heavy metal contamination (Fe) and phytoestrogen which acts as endocrine disruptors. **Georgescu et al. (2011)** denoted that heavy metal exposure results in disturbed fertility in females, as revealed by an in vitro study that examined the consequences of Pb and Fe exposure on cytochrome P-450 aromatase (P-450 ARO) and ß-estradiol receptors, two key proteins in the function of the pituitary-ovarian axis. It was shown that the activity of both P-450 ARO and ER-β in the granulosa cells of the ovarian follicles was strongly inhibited. Also , they reported that Cd and Fe were able to potentiate the estradiol-induced response in a dose-dependent manner, thus indicating that Fe can act as a potential endocrine disruptor by modulating the estrogenic activity of endogenous hormones (xenoestrogen) .Concerning the phytoestrogen effect on increasing the level of estrogen ,**Joffe (2003)** noticed reduced fertility in Australian ewes due to the clover that they were grazing. This condition, which came to be known as 'Clover Disease', was traced to phytoestrogens—plant compounds that have estrogenic properties—in the clover. Ewes feeding on Australian clover developed abnormal plasma concentrations of endogenous hormones, with subsequent reduced fertility . In addition ,**Adams (1990)** reported that in Australia, more than 1 million ewes have permanently damaged reproductive tracts because they have been grazed on estrogenic pasture. These effects occur in the absence of classical clinical "clover disease". The lesions result from an "organisational" action of estrogen, causing a mild sexual transdifferentiation to occur in ewes during adult life, with the main lesion being found in the cervix .

The reason for the increase in progesterone in this study is not clearly known and may be considered to be either of ovarian or adrenal origin . Moreover, low level of nutrition is associated with rapid higher level of progesterone, especially after mating and lower rate of embryo survival due to low metabolic clearance rate (**Ahmed ,2007) .**

The increase and decrease in the levels of testosterone in G2andG3,respectively may be due to heavy metal and phytoestrogen (isoflavones) disrupting effect on 5 alpha-reductase, which is involved in the conversion of testosterone to dihydrotestosterone **(Yi et al. ,2002) .**

There are many endocrine-disrupting chemicals that can interfere with thyroid function by acting on different points of regulation of thyroid hormone synthesis, release, transport through the blood, metabolism of thyroid hormone, and thyroid hormone clearance . In addition, many natural substances are known to affect thyroid function, including low iodine as well as goitrogens in various foods **(Diamanti-Kandarakis et al. ,2009).**

Results recorded in table (5) declared that there was a significant($P\leq 0.05$) decrease in T3 levels and insignificant increase in T4 levels in the investigated infertile groups comparing with the control group .TSH levels showed significant($P\leq 0.05$) increase in G2 and insignificant increase in G3 compared to the control group .The decrease in T3 and increase in TSH levels may be due to the inhibitory effect of phytoestrogen (isoflavones) on thyroperoxidase , a heme – containing enzyme , that controlled the oxidation of iodine into iodide which is the form of iodine that enters the thyroid cells to form thyroglobulin **(Doerge and Chang ,2002) .** In addition , **Tuckova et al. (1995)** believed that environmental pollution may cause decrease in the output of thyroid hormones in serum .Also, the decrease in T3 levels may be due to zinc deficiency recorded in these animals as it has been reported that zinc participate in protein synthesis and essential for thyroid function since involved in T3 binding to its nuclear receptor **(Liu et al., 2001) .** The reason for an increase in T4 and a decrease in T3, which were observed might have been due to a slowdown in the conversion of T4 to T3 in peripheral tissues. This may be due to the effect of endocrine-disrupting chemicals

(EDCs , as iron and phytoestrogens) that limits the conversion of T4 into T3 by causing the changes, mainly in 5.-deiodinase as well as malic enzyme and 6-phosphogluconate dehydrogenase activities **(Diamanti - Kandarakis et al. ,2009).**

Conclusion

Disturbance of ovarian activity is the most prominent cause of reproductive failure and economic losses in farm animals. Currently a lot of factors negatively affect ovarian activity in farm animals, especially in the developing countries. This study indicated that heavy metals pollution of well-water and berseem fodder with iron as well as serum zinc ,copper and phosphorus deficiency affect fertility of ewes at El- Dakhla City farms . Moreover , grassing on berseem clover (*Trifolium alexandrinum* , Al- Abbd) may be the cause of reduced ovulation rate owing to high content of phytoestrogen .So, iron and phytoestrogen act as endocrine disrupting compounds which affect gonadotrophic hormones (LH&FSH), steroidogenesis and thyroid hormones production that affect ewes fertility .

Recommendations

In order to keep ovarian activity at the maximum possible level to obtain the peak fertility and benefits from farm animals breeding, the following points should be considered :

1-Provision of adequate, balanced nutrition and dietary supplementation with fat and minerals mixture especially during the critical periods in the animal life. Prepubertal and postpartum periods should be supplied with the balanced diet to ameliorate the limitation of intakes and to improve the conception rate .

2- It could recommend that biogen-zinc could be added to ewes ration at the levels of 0.5 or 1.0 g/head/day during reproductive stages in order to improve reproductive performance by increasing the incidence of estrus, conception rate and fertility .

3-Provision of treated well-water to avoid the effect of pollution with iron on reproductive performance of the ewes.

4- Effects of low concentrations of phytoestrogens on reproductive function in ruminants are likely to receive increasing attention.

5- To ameliorate the effect of grazing sheep in pastures high in phytoestrogen levels (berseem clover) , the animals could be treated with Se to increase the conception rate .

6-Rams in these farms used for mating should be examined to pinpoint the effect of endocrine disrupting compounds (iron & phytoestrogen) and mineral deficiency on their reproductive performance.

Table (1) :Some heavy metals and calcium concentration (ppm) in well-water at El -Dakhla sheep farms

Parameter	Minimum	Maximum	Mean ± SE	MPL of WHO (ppm,mg/l)	MPL of EMH (1995) (ppm, mg/l)
Iron	**13.615**	**17.661**	**15.638± 0.640******	**0.3[a]**	**0.3**
Lead	**0.002**	**0.006**	**0.004 ± 0.001****	**0.05[b]**	**0.05**
Cadmium	**0.001**	**0.005**	**0.003 ± 0.001****	**0.01[c]**	**0.005**
Calcium	**50.200**	**57.000**	**53.600± 1.281****	**75[d]**	**-**

EMH :Egyptian Ministry of Health(1995) . a & d :WHO (2002), b & c :WHO (2003). MPL :Maximum permissible limit. Data are expressed as means ± SE of five samples.
***: Significantly different from the maximum permissible limits at P≤0.0001.
**: Significantly different from the maximum permissible limits at P ≤0.001.

Table (2) : Some heavy metal concentration (ppm) in berseem fodder at El- Dakhla City

Parameter	Minimum	Maximum	Mean ± SE	MPL (ppm, mg/kg)
Iron	**5.050**	**6.890**	**5.970 ± 0.393*****	**0.3[a]**
Lead	**0.010**	**0.016**	**0.013 ± 0.001****	**0.3[b]**
Cadmium	**0.007**	**0.015**	**0.011 ± 0.002****	**0.2[c]**

a:WHO (1999) , b & c : European Union (EU) food standards (EC ,2006). MPL:Maximum permissible limit. Data are expressed as means ± SE of five samples.
***: Significantly different from the maximum permissible limits at P ≤0.0001. **:Significantly different from the maximum permissible limits at P≤0.001.

Table (3): Serum concentration of some heavy metals , trace elements and mineral of the control (G1) and infertile ewes (G2&G3) at El -Dakhla City .

Groups / Parameters	G1	G2	G3
Iron (ppm)	**4.708 ± 0.315[c]**	**6.188 ± 0.333[b]**	**10.249 ± 0.250[a]**
Lead (ppm)		**ND**	
Cadmium(ppm)		**ND**	
Zinc (ppm)	**1.147 ± 0.080[a]**	**0.428 ± 0.370[c]**	**0.699 ± 0.040[b]**
Copper (ppm)	**0.288 ± 0.020[a]**	**0.079 ± 0.010[b]**	**0.248 ± 0.030[a]**
Calcium (mg/dl)	**10.930 ± 0.320[a]**	**11.900 ± 0.520[a]**	**12.140 ± 0.410[a]**
Inorganic phosphorus(mg/dl)	**7.140 ± 0.250[a]**	**7.480 ± 0.350[a]**	**5.780 ± 0.300[b]**

Data are expressed as means ± SE of ten samples .

ND :not detected.

[a,b,c] Means within the same raw with different superscripts are significantly different at ($P \leq 0.05$) .

Table (4) : Serum levels of reproductive hormones in the control (G1) and infertile ewes (G2&G3) at El - Dakhla City .

Groups / Parameters	G1	G2	G3
LH (ng/ml)	**10.230 ± 0.360[a]**	**9.190 ± 0.470[a]**	**7.670 ± 0.377[b]**
FSH (ng/ml)	**9.690 ± 0.390[a]**	**7.790 ± 0.480[b]**	**7.740 ± 0.620[b]**
Estradiol (pg/ml)	**86.840 ± 4.590[c]**	**194.460 ± 33.210[a]**	**120.000 ± 10.230[b]**
Progesterone(ng/ml)	**8.990 ± 0.252[c]**	**16.250 ± 0.356[b]**	**23.670 ± 0.402[a]**
Testosterone(ng/ml)	**1.460 ± 0.070[b]**	**2.660 ± 0.550[a]**	**0.870 ± 0.147[c]**

Data are expressed as means ± SE of ten samples .

[a,b,c] Means within the same raw with different superscripts are significantly different at ($P \leq 0.05$) .

Table (5) : Serum Triiodothyronine (T3), thyroxin (T4) and thyroid stimulating hormone (TSH) of the control (G1) and infertile ewes (G2&G3) at El - Dakhla City .

Groups / Parameters	G1	G2	G3
T3	**4.950 ± 0.145[a]**	**2.750 ± 0.158[b]**	**2.210 ± 0.266[b]**
T4	**10.200 ± 0.530[a]**	**12.200 ± 0.339[a]**	**12.050 ± 1.400[a]**
TSH	**0.125 ± 0.012[b]**	**0.165 ± 0.009[a]**	**0.146 ± 0.013[ab]**

Data are expressed as means ± SE of ten samples.

[a,b] Means within the same raw with different superscripts are significantly different at ($P \leq 0.05$) .

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