METHODOLOGY (METODOLOGIA)

Apoptotic factors in testicular cells of chickens

Fatores apoptóticos nas células testiculares de frango

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Fertility is the first and most important requisite of poultry breeding. The number of fertile eggs produced for hatching dictates the ultimate profitability of hens. Infertility is a major economic loss in poultry industry (HEYDARI *ET AL.* 2015). Although males and females both contribute to decreasing fertility, however, low fertility is thought to be largely a problem in males because the ratio of males to females in a flock is very low (OMMATI *ET AL.*, 2013). The deterioration in fertilizing ability has been attributed to many factors like age, weight and decline in semen quality (KHAN, 2011). Testis are the chief reproductive organs in the males; they perform the two functions: spermatogenesis and steroidogenesis. Spermatogenesis occurs in the germinal epithelium of the seminiferous tubules and steroidogenesis is done by interstitial cells (Leydig cells), which are controlled by gonadotropins (MATHUR AND D'CRUZ, 2011). Testis are important reproductive organs of chickens, which are related to the production and reproduction of the poultry industry. If the testicles cause poisoning, the quality of sperm in the testis will be affected, eventually

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leading to sperm dysplasia or deformity, which will affect the success rate of mating. Or even if the mating is successful, the offspring will be stunted, which will affect the meat quality and harm the development of the breeding industry (Shao *et al.* 2018). Aberrant sperm production causes infertility, they by will not to be able to conceive the female partner (Siva Kumar & Neeraja, 2019). Oxidative stress is the major factor for the infertility, tension in the testis.Several factors like; exposure to endocrine disruptors, physiological stress, temperature or pH imbalance and present life style are also major reasons for infertility in males (Darbandi *et al.* 2018; Rehman *et al.* 2018).

Apoptosis and autophagy are two major pathways of programmed cell death under developmental and/or stressful conditions, and they are closely related (Bongaerts, 2008). Apoptosis can be triggered via the extrinsic pathway, which is activated through Tumor Necrosis Factor (TNF) and Factor associated suicide (Fas), or the intrinsic pathway, which is mediated by B cell lymphoma/leukemia 2 (Bcl-2) gene family releasing. Moreover, both pathways induce apoptosis through activation of effector caspases (Yang *et al.*, 2017). Testicular cell populations, *i.e.*, Sertoli cells, spermatogonia, and spermatids, are highly regulated in terms of their number and viability. Throughout the breeding period of the male, many changes occur in this regard previously, it was believed that regression of the testis was due to necrotic processes brought about by the failure of hormones to maintain the integrity of the seminiferous epithelium. However, recent studies, again mostly with mammals, have shown that testicular cell death is not random, but is initiated by highly regulated cell suicide mechanisms instigated by a series of intracellular protein-protein interactions mediated by signaling molecules such as the death effector domain (Thurston & Korn, 2000). A novel death effector domain, identified as death effector domain-containing testicular molecule, has been shown to be highly expressed in human and rat testis (Leo *et al.*, 1998). Control of cell death by this method is called apoptosis, and includes loss of membrane asymmetry, condensation of the cytoplasm and nucleus, and internucleosomal cleavage of DNA. Microscopically, cells marked for apoptosis can be identified by their unusually small, pyknotic nuclei. Cell death is mediated by a series of cytosolic proteases called caspases and endonucleases that cleave the DNA (Thurston & Korn, 2000). Testicular apoptosis appears to be by the Fas-ligand, Fas-receptor method. Binding of Fasligand to cells expressing Fas-receptor proteins initiates apoptotic-signaling pathways. Sertoli cells appear to express genes that produce Fas-ligand when promoted by the universal transcriptional activator Sp1 (TM4 Sertoli cell line, McClure *et al.*, 1999). The Fas-

ligand, Fas receptor mechanism has been implicated as being responsible for maintaining the immune privilege of the testis and in regulating germ cell apoptosis (human; PENTIKAINEN *ET AL*., 1999). In the rat, physiological apoptosis is believed to occur continuously to control the size of the germ cell population. Injury to Sertoli cells causes Fas-ligand upregulation to eliminate Fas receptor-positive germ cells that no longer can be supported (LEE *ET AL.*, 1999). There is much evidence that Sertoli cells participate in paracrine control of germ cell function by Fas-mediated pathways (LEE *ET AL.*, 1997).

Sertoli cells in testicular stroma are directly affected by certain environmental conditions or toxins. In male reproductive injuries caused by chemical and physical factors, the damage to the structure and function o Sertoli cells are all earlier than those of other tissues (RICHBURG & BOEKELHEIDE, 1996). The number and functional status of Sertoli cells determine the testicular size and the normal progress of spermatogenesis, playing a decisive role in male reproductive function (CHEN *ET AL*., 2015). Proteins such as bcl-2, an oncogene-derived protein, can confer negative control in the pathway of cellular suicide (BASU & HALDAR, 1998). VILAGRASSA *ET AL*. (1997) found that bcl-2 and bcl-x were produced during spermatogenesis in chicken testis, and stated that differential expression of bcl-2 and bcl-x is consistent with the reported different susceptibility to apoptosis of spermatogonia, meiotic cells, and postmeiotic cells (THURSTON & KORN, 2000). Plasma membrane lipids play a significant role in sperm fertilizing capacity. Avian sperm cell membranes have a much greater concentration of polyunsaturated fatty acids (PUFAs) than mammalian sperm cells and are therefore more susceptible to lipid peroxidation (LPO) in the presence of reactive oxygen species (ROS) (HEYDARI *ET AL.,* 2015).

GENERAL MECHANISMS OF APOPTOSIS

Programmed cell death or apoptosis has a vital role in various biological events. The elimination of unwanted or damaged cells by apoptosis is indispensable for embryogenesis, maintaining cellular homeostasis, and normal regulation of the immune system (LESSENE *ET AL.*, 2008). Because impaired apoptosis induces abnormal cell proliferation, the dysregulation of cell death mechanisms is a major hallmark of cancer development (HANAHAN & WEINBERG, 2000). Programmed cell death is activated by intracellular stresses and developmental cues. Well-known representative intrinsic regulators, the extended BCL2 family proteins play crucial roles in cell death regulation and are able to regulate several cell death mechanisms, including apoptosis, necrosis and autophagy (LEVINE & KROEMER, 2008; REED, 2008). BCL2 family proteins contain at least one

of four BCL2 homology (BH) domains (i.e., BH1, BH2, BH3 or BH4), and the number of BH domains included in proteins is associated with its apoptotic functions (Yip & Reed, 2008). Antiapoptotic BCL2 family members (e.g., BCL2, BCL2L1, MCL1 and BCL2A1) have all four BH domains. Proapoptotic BCL2 family members are categorized into two groups: the multiple-BH domain, which includes members BAX, BAK1 and BOK, and a single BH-3 group that includes members BID, BIM, BAD and NOXA.

The antiapoptotic BCL_2 family prevents endoplasmic reticulum (ER)-mediated cell death by reducing basal Ca_2+ concentrations in the ER for regulation of cell survival (Xu *et al*., 2005), whereas proapoptotic family members like BAX and BAK induce ER-mediated apoptosis by inhibiting the effect of antiapoptotic members or by opposing effects on ER Ca2+ concentrations for regulation of cell death (Yip & Reed, 2008). Proapoptotic BCL2 family proteins, such as BAX and BID, stimulate mitochondrial outer membrane permeabilization (MOMP) that induces the release of regulators or activators (such as caspase activator) and other cell-death mediators. In contrast, antiapoptotic family members, such as BCL2 and BCL2L1, guard the outer membrane and conserve its integrity by the action of BAX and BAK (Yip & Reed, 2008). There is a close association between tumor development, initiation and resistance to chemotherapy and the dysfunction of BCL2 family proteins (Lessene *et al.,* 2008). Cysteine aspartate-specific proteases (CASPs or caspases) serve as intrinsic initiators of apoptosis by cleaving substrates at aspirate residues (Kim *et al*., 2009). The caspase protein family currently consists of 13 and 11 isoenzymes in mammals and humans, respectively (Nicholson, 1999). The function of caspases is closely connected to initiation and execution of apoptosis, with caspases categorized into either initiator or effector caspases. CASP2, CASP8, CASP9 and CASP10 are initiator caspases, and CASP3, CASP6 and CASP7 are effector caspases (Kumar, 1999). Known as interleukin 1ß converting enzyme, CASP1 plays an important role in both apoptosis and inflammation (Feng *et al*., 2005). Apoptosis is regulated by stepwise activation of caspases for the processing or cleaving of other caspases (Slee *et al.,* 1999). Therefore, caspases are proapoptotic proteins that are likely to serve as tumor suppressors, and their dysregulation is involved in the initiation and development of tumors.

Testicular apoptosis of insulin sensitiser metformin

Metformin, an insulin sensitiser, is known to mimic food restriction and may modulate male fertility. Tartarin *et al*., (2012) showed a decrease in testis size by 1 day postpartum after embryonic exposure of

mice to metformin. Moreover, it appears that metformin decreases testosterone secretion in human and mouse testis in organotypic culture. Faure *et al. (*2016) showed that in vitro metformin exposure was associated with reduced cellular proliferation and variations in the secretory ability of Sertoli cells. Moreover, they demonstrated a negative effect of metformin exposure on the germ cell population. In vivo, metformin exposure induced a decrease in testis weight and in spermatid production, suggesting that 3 weeks of metformin administration in drinking water is sufficient to delay spermatogenesis (Faure *et al*., 2016). Testosterone levels were also reduced by 50% after metformin administration in vivo (Faure *et al.,* 2016). Testosterone is known to be crucial for spermatogenesis. These results are concordant with those obtained in mammals (Tartarin *et al.*, 2012), suggesting a conservation of this mechanism in birds. In primary rat Leydig cell culture, the use of a natural AMPK ligand (curcumin; Zang *et al.,* 2006) decreases testosterone secretion through a reduction in cholesterol transport into the mitochondria and decreased conversion of progesterone into androstenedione (Svechnikov *et al.,* 2009).

Heat stress-induced testicular apoptosis

The accepted thermo-neutral (TN) ambient temperature ranges from 14 to 25º C for poultry. The animals feel comfortable themselves in this temperature intervals. When the ambient temperature exceeds the upper limit of TN zone values, heat stress (HS), which is called as the deterioration of balance between the body temperature and heat thrown out from the animal's body, is occurred (Nardone *et al.,* 2010). HS begins when the ambient temperature becomes higher than 27 Cº and is readily apparent above 30º C (Ezzat *et al.,* 2011). HS-related failures on productivity of poultry range from reduced growth rate (Çiftçi *et al.,* 2013), reduced feed intake (Tonbak & Çiftçi, 2012), reduced feed efficiency (Orhan *et al*., 2012), reduced carcass weight (Çiftçi *et al*., 2013) and reduced egg quality (Sahin *et al.,* 2008). In addition, the detrimental effects of HS on reproductive features of male poultry have been reported to be decreases in testis weight (McDaniel *et al.,* 2004), sperm count, sperm motility, and increase in dead sperm rate (Ebeid, 2012). HS has been reported to cause the loss of spermatogenic cells and degenerative alterations in testis of broilers (Terim Kapakýn *et al.*, 2013), the vacuolisation of germinal epithelium, multinucleated giant cell formations in mice (Yin *et al.,* 1997), the degeneration in seminiferous tubules and the spermatogenic arrest in developing lambs (Rasooli et al. 2010) and adult llamas (Schwalm et al., 2007).

In the HS group in chicks, the integrity of the testicular structure was damaged significantly, and the seminiferous epithelium became thinner with vacuolar-like changes; the spermatogonia also showed a decreased number and loose and disordered arrangement (Chen *et al.*, 2015). These data suggested that HS might have damaged the structure and function of the stromal Sertoli cells in testis of chicks in the HS group and thereby caused the thickening of seminiferous tubular epithelium and the inhibition of the development of different levels of spermatogenic cells, resulting in the phenomena of dysplasia exemplified by various degrees of atrophy (Chen *et al.,* 2015). The physiological mechanisms that result in HS-induced reproductive failure in poultry are not completely understood, but most logically explained by increased intra-testicular temperature results from increased body temperature (Türk *et al.* 2015), and lipid peroxidation due to the accelerated metabolic rates under stress conditions (Ebeid, 2012).

The cell membrane of avian spermatozoa contains high amounts of polyunsaturated fatty acids (PUFAs). The high levels of PUFAs render avian spermatozoa vulnerable to lipid peroxidation, which is considered to be an important factor for male reproductive dysfunction, and therefore these cells require adequate antioxidant capacities (Türk *et al.* 2015). The precise balance between generation of reactive oxygen species (ROS) and antioxidant defence system, which is capable of protecting against free radicals and toxic products of their metabolism, is considered to be an important determinant for semen quality in avian species (Surai *et al*. 2001). Furthermore, HS significantly reduces the marker enzymes of intestinal absorption such as disaccharidases, alkaline phosphatase (AKPase), and adenosine triphosphatase (ATPase), causing dysfunction of the intestinal mucosal antioxidant system in poultry (Chen *et al.,* 2013, 2014).

Furthermore, under HS, the amounts of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estradiol (E2) declined, and the progesterone (P4) level significantly reduced, too, after an initial elevation (Li and Cui, 2013). Because normally secreted LH and FSH in male animals promote the development of the seminiferous epithelium and the proliferation of spermatogonia, abnormal levels of LH and FSH secretion ultimately impair the normal process of testicular development (Chen *et al*. 2015).

The Bax (apoptotic) and Bcl-2 (anti-apoptotic) proteins exist in the culmination of apoptosis after the onset of cellular stress. The ratio of these molecules has been implicated to be a critical determinant of cell fate, such that elevated Bcl-2 favours extended survival of cells and

increasing levels of Bax expression accelerates cell death (Sinha Hikim & Swerdloff, 1999). Apoptosis is also an indicator of DNA damage in the cells including testicular germ cells, and an increase in free radicals results in increased testicular apoptotic germ cell (Maheshwari *et al.,* 2009). In the study of Türk *et al.* (2015), HS caused significant increases in the density of testicular Bax immunpositivity, Bax/Bcl-2 ratio and significant decreases in the density of testicular Bcl-2 immunpositivity of quails as compared to TN conditions. It has been reported that HS-induced overproduction of free radicals causes sperm DNA damage and testicular apoptosis (Durairajanayagam *et al.,* 2014).

Toxico-Pathological Effects of Cottonseed Meal (gossypol)

Cottonseed meal (CSM) is a by-product obtained after oil extraction from cottonseeds (Nagalakshmi *et al.*, 2007). Incorporation of CSM in poultry feed is limited due to presence of gossypol which is a biologically active terpenoid aldehyde mainly present within the puncta or 'glands' of cotton seeds. Free gossypol is toxic and chemically reactive. When free gossypol covalently binds to amino acids mainly lysine, it becomes non-toxic and is known as bound gossypol (Mahmood *et al.*, 2011). The availability of lysine in CSM is less because the free gossypol binds with lysine in meal during processing resulting in bound gossypol (Ryan *et al.*, 1986). Feeding of pigment glands in chicken led to depressed weight gain, decreased feed efficiency, and increased mortality (Smith, 1970). Gossypol, as an anti-fertility agent has previously been reported in different species including rat (Singh & Rath, 1990), rabbit (Saksena *et al.*, 1981) and domestic fowl (Kalla *et al.*, 1990). Intramuscular injection of purified gossypol and gossypol acetic acid at 25 mg/kg in male quails showed testicular atrophy and drastic reduction in size of androgen-dependent cloacal gland (Lin *et al.*, 1988). Subcutaneous injection of 25.0 mg free gossypol/kg b wt in rats decreased the weight of different secondary sex organs (Gafvels *et al.*, 1984). In broiler breeder males fed 20 and 30% CSM (daily free gossypol ingestion 4.56-7.17 mg/kg b wt) showed a significant decrease in absolute and relative weight and volume of testis along with reduction in semen volume and sperm counts which These changes have direct relation with pathomorphological alterations in testis and low serum testosterone concentration (Mahmood *et al.,* 2011). Moreover, Histopathological alterations in testis of male chickens given feed containing 30 % CSM included increased inter-tubular connective tissue proliferation, presence of necrotic spermatids in the tubular epithelial layer and absence of spermatozoa (Mahmood *et al.,* 2011).

JATROPHA CURCAS MEAL (PHORBOL ESTERS) INDUCED TESTICULAR APOPTOSIS

Jatropha curcas meal (JCM), obtained after oil extraction, has been characterized as a potential animal feedstuff due to its high crude protein (CP) content (MAKKAR *ET AL.*, 2008) and high levels of essential amino acids, except for lysine (MAKKAR *ET AL*., 1998; RAKSHIT & BHAGYA, 2008). However, its toxicity, mainly attributed to phorbol esters (PE), has hindered its use as animal feed (MAKKAR *ET AL.,* 1997). EL-BADWI & ADAM (1992) and EL-BADWI ET AL. (1995) reported mortality and severe pathological changes in Brown Hisex chicks. The histological examination of the testis reinforces the direct detrimental effect of *Jatropha* incorporation on the sexual development of the broilers (BARROS *ET AL.,* 2015). In this study, while the testis from control group were well developed, the testis of birds fed diets containing JCM were atrophic, with a decrease in the size of seminiferous tubules, which were small and lined with inactive Sertoli cells and rare spermatogonia. A wide range of biological effects, including tumor promotion and inflammation, are attributed to PE, and these adverse effects are highly structure-specific (BALDINI *ET AL.,* 2012).

MYCOTOXINS (ZEARALENONE)**:**

The worldwide contamination of raw materials with mycotoxins is a safety concern in both humans and in animal species, including poultry. In Europe, the occurrence of aflatoxins and ochratoxins is relatively low. The co-occurrence of mycotoxins produced by *Fusarium* (Fusariotoxins, FUS) in avian feed is the most frequent (GUERRE, 2016; KOSICKI *ET AL.* 2016). Among FUS, four compounds are subject to international and European guidelines on their maximum tolerated levels in avian feed. These toxins are members of group A and B trichothecenes, T-2 toxin (T-2) and deoxynivalenol (DON) respectively, the myco-estrogenic zearalenone (ZON), and fumonisins B1 and B2 (FB1 and FB2), belonging to group B of fumonisins (FB). Among the FUS with regulatory limits, DON, FB and ZON occur the most frequently in poultry diets, whereas T-2 is less frequent in Europe (PINOTTI *ET AL.,* 2016; Zachariasova et al. 2014). Efects of FUS on reproductive function are usually not reported in avian species, except wi*th very high levels of ZON* (MURUGESAN *ET AL.,* 2015; CORTINOVIS *ET AL*., 2013). The weights of testis were significantly reduced in broilers fed 200 to 400 mg ZON/kg (ALLEN *ET AL.,* 1981). In mammals, the most toxic e-ect of FUS other than ZON on reproductive function has been mild-to-moderate lesions of testis with Sertoli cell degeneration and impaired spermatogenesis observed in rabbits fed 0.13 to 5 mg FB1/kg diet for 175 days (EWUOLA & EGBUNIKE, 2010a,b). Studies conducted in mice suggested that DON may have an adverse effect on the epididymal weight at 10 mg/kg of feed for 90 days with slight changes

in relative testis weight and spermatid counts, but no histological changes (EFSA, 2015). In the study of METAYER *ET AL.* (2019) , no significant difference was observed in the variables measured to reveal toxicity of FUS in broiler testis except a decrease in the diameter of the seminiferous tubule and a decrease in catalase activity in the DONFBZON group that are dificult to interpret.

LEAD (PB) PB-INDUCED AUTOPHAGY AND APOPTOSIS IN THE CHICKEN TESTIS

Lead (Pb) is a toxic environmental pollutant. Its degradation or elimination is very difficult. The misuse of Pb ammunitions in hunting (GIL-SANCHEZ *ET AL.,* 2018) and leaded gasoline (ABDENNADHER *ET AL.,* 2010) caused Pb pollution which was a threat to wild birds. Pb can enter the body through foraging, keep accumulating, and cause Pb poisoning in wild birds. Millions of birds died annually from Pb poisoning worldwide (DE FRANCISCO *ET AL.,* 2003).

More importantly, Pb toxicity affects reproductive function of birds. Studies had shown that Pb pollution caused the decline of bird breeding capacity in northern Sweden (BERGLUND *ET AL.,* 2010) and reduced sperm motility in red-legged partridges (VALLVERDÚ-COLL *ET AL.,* 2016). Pb could accumulate in chicken testis (HUANG *ET AL*., 2017b). Many researchers found that H_2O_2, CAT, TAOC, GSH, and SOD were oxidative stress indicators. Pb poisoning can decrease CAT and TAOC activities, and cause oxidative stress in rat livers (KHALIL *ET AL.,* 2018). H_2O_2 content increased, CAT and SOD activities and GSH content decreased, and oxidative stress occurred in Pb-treated fungus cells (HUANG ET AL., 2017a). Excess Pb can decrease CAT activity and cause oxidative stress in rat testis (DKHIL ET AL., 2016). Pb caused oxidative stress through decreasing GSH content and CAT activity in rat testis (HASANEIN *ET AL.,* 2018). Mabrouk reported that Pb decrease CAT and SOD activities and GSH content, and cause oxidative stress in rat testis (Mabrouk and Ben Cheikh, 2015). HUANG *ET AL.* (2019) found that Pb increased H_2O_2 content; decreased CAT, TAOC, and SOD activities in the chicken testis. Additionally, these researcher also observed the microstructure of chicken testis and found testicular damage in the Pb group and concluded that Pb caused damage and oxidative stress in the chicken testis (HUANG *ET AL*,. 2019). Some reports indicated that autophagy was associated with oxidative stress. CHEN *ET AL.* (2014) found that H2O2 decreased mTOR and induced autophagy in nucleus pulposus cells. Copper caused oxidative stress through decreasing CAT activity; and caused autophagy through increasing mRNA expression of ATG5, Beclin1, Dynein, LC3-I, and LC3-a! in chicken hepatocytes (YANG *ET AL*., 2018). In addition, previous studies found that Pb poisoning could induce autophagy. Pb promoted protein

levels of Beclin1, LC3-I, and LC3-II; and induced autophagy in the hippocampus of rats (Zhang et al., 2012). Han *et al.* . (2017) reported that Pb increased mRNA and protein levels of ATG5, Beclin-1, Dynein, LC3-I, and LC3-II; decreased mRNA and protein levels of mTOR; and induced autophagy in chicken spleens. Huang *et al.* (2019) found that Pb treatment promoted mRNA and protein expression of Beclin 1, Dynein, ATG 5, LC3-I, and LC3-II; and inhibited mRNA and protein expression of the mTOR. Our result meant that Pb induced autophagy in the chicken testis. Further more, we also observed the ultrastructure of chicken testis.

Manganese-induced testicular toxicity:

Manganese (Mn) is a naturally occurring trace metal commonly found in the environment. It is essential in maintaining the proper function and regulation of many biochemical and cellular reactions (Takeda, 2003). While Mn intoxication caused by prolonged exposure produces a severe and debilitating disorder known as maganism (Liu *et al*., 2013). Thus, reports of wild birds being affected by Mn toxicity are common. Piscivorous birds, such as herons and egrets (family Ardeidae), are suitable indicator organisms of environmental pollution in aquatic systems (Sakellarides *et al*., 2006). Manganism can disturb the balance of trace elements in immune organs and induce immune suppression at the molecular level (Liu *et al.*, 2012). However, other studies have shown that heavy metals can also influence the reproduction and general health of some birds (Janssen *et al*., 2003; Dauwe *et al*., 2004). As an environmental toxicant, Mn damages the central nervous system and has procreant toxicity. Mn can also be transplacental, affecting embryos and the growth of offspring (Zhang *et al*., 1998; Spencer, 1999). Numerous reports have shown that divalent metal ions are a contributing factor in the acute toxicity of peroxides in laboratory animals (Jomova *et al*., 2010). Heavy metals are important regulators of cell apoptosis. Heavy metals have high affinity for thiol-group-containing enzymes and proteins, which are responsible for normal cellular defense mechanisms (Gong *et al*., 2008). Long-term exposure to heavy metals could lead to apoptosis (Eichler *et al.,* 2006). Mn is a potent inducer of apoptosis in different cell types, including human B cells (El Mchichi *et al*., 2007), PC12 cells (Hirata, 2002), NIH3T3 cells (Oubrahim *et al.*, 2002), and SH-SY5Y neuroblastoma cells (Li *et al*., 2010). However, the precise mechanisms that mediate such effects are not well defined (El Mchichi *et al*., 2007). High dietary Mn contentsin chicken led to its accumulation in the testicles of cocks, and Mn most likely crossed the blood-testis barrier (BTB) (Liu *et al.*, 2013). Testicular changes in response to Mn toxicity have been observed in a variety of animal models. Animal experimental studies have

indicated toxicity to testicles in rabbits after intra-tracheal administration of Mn oxide (Chandra *et al*., 1973). Oral administration of Mn chloride was reported to produce histopathological testis damage in monkeys (Murth *et al.*, 1980) In addition, Mn sulfate injection in rats for 25 d produced degeneration in the seminiferous epithelium, a depleted number of spermatids, and no spermatocytes in the seminiferous tubules (Ponnapakkam *et al.,* 2003). Liu *et al.* (2013) demonstrated that dietary Mn induced similar histopathological changes in the chicken testicles (edema, necrosis, degeneration of seminiferous tubules) and apoptosis in the spermiogenic region of the seminiferous epithelium.

Cells survive the oxidative stress induced by heavy metals due to the action of intricating antioxidative systems, comprising both oxidants and antioxidants. The antioxidant defense systems include radical scavenging enzymes, such as SOD and GPx. SOD is involved in protective mechanisms in tissue injury following oxidation and phagocytosis. Wirth et al. (2007) reported that the activities of SOD and GPx in rat tests were obviously lower than those in the control group and that the apoptosis of spermatogenic cells appeared 4 weeks after intraperitoneal injection with 15 mg kg–1 and 30 mg kg–1 MnC_{l2}. Zhang *et al.* (2002) indicated that newborn mouse brain cells were exposed to 2—0–4 mol L–1 Mn in vitro, which corresponded to a clear decrease in the activities of SOD and GPx in the cells and a significant increase in MDA contents. In the present study, the activities of SOD and GPx decreased but the MDA contents increased gradually along with increasing long-term exposure to dietary Mn. These results were similar to previous reports, illustrating that cellular lipid peroxidation was enhanced by exposure to Mn. The activities of GPx and SOD were weakened, whereas the MDA contents in testicular organs increased. It was indicated that the total antioxidative capability of chicken testicular organs in the Mn-diet-fed group was most likely impaired, based on the decreased antioxidant ability (Liu *et al.,* 2013).

The apoptosis in arsenic-induced oxidative stress:

Arsenic (As), which has been studied for centuries, is abundant in the earth's crust, soil, water, almost all tissues of animals and plants and is an important topic in both mainstream media and the scientific literature (Martinez *et al*., 2011). Arsenic is classified as a class I carcinogen by the international agency for research on cancer (IARC) and specified the critical value of drinking water as 10 ìg/L by the World Health Organization (WHO) and the U.S. Environmental Protection Agency (EPA) (Brandon *et al.*, 2014). Numerous reports have shown that chronic arsenic trioxide (As_2O_3) exposure can cause the damages of the

organisms, i.e., skins, lungs and bladder cancers, cardiovascular dysfunction, adverse pregnancy outcomes, cognitive deficits and type-2 diabetes (Abdul *et al*., 2015). Zhao *et al.* (2017b) have proved that exposure to arsenic trioxide (As_2O_3) can cause neurotoxicity in chickens. Dietary As_2O_3 can trigger apoptosis in immune organs of chickens (Zhao *et al*., 2017a). Previous researches have suggested that As2O3 induced testicular toxicity that can cause inflammatory and heat shock responses in chickens (Sun *et al*., 2017b).

Oxidative stress is part of the main mechanisms of inorganic arsenic (iAs) poisoning (Gao *et al.*, 2013). Sodium arsenite ($NaAsO_2$) exposure toxicity can stimulate reactive oxygen species (ROS) and cause oxidative stress by affecting the balance between the prooxidant and the antioxidant in the body (Samuel *et al*., 2005). As_2O_3 could trigger extrinsic and intrinsic apoptosis pathways in immune organs of chickens, meanwhile, ROS generated by oxidative stress might be an important driver of excessive apoptosis (Zhao *et al.*, 2017a). In chicken, vacuolar and degenerative spermatogenic cells and apoptotic cells in the As_2O_3 treatment groups was reported which meant the degeneration and necrosis of cells and the decline of the testicular function (Shao *et al.,* 2018). Furthermore, these authors reported taht antioxidant enzyme activities in the testis of chickens varied with As_2O_3 concentrations and duration of As_2O_3 exposure. Likewise, a significantly dosedependent decrease of GSH was found during the exposure (Shao *et al.,* 2018). In chickens, the activities of antioxidant enzymes, such as GPx and CAT, were inhibited after As_2O_3 exposure depending on exposure time and concentration in immune organs and brain tissues (Zhao *et al*., 2017a; Zhao *et al.*, 2017b).

Apoptosis induced by $NaAsO_2$ depends on exposure time and concentration (Chen *et al*., 1998). In chicken testis, As_2O_3-induced apoptosis was mediated through the increases of caspases activation, p53 and the corresponding changes of Bcl-2 gene family (Shao *et al.*, 2018). It is reported that oxidative stress may trigger apoptosis (Xie *et al*., 2017). Oxidative stress accumulates too much — OH that cannot be cleared by the body later they cause DNA damage. What's more, DNA damage can increase the level of p53 which can regulate gene expression of Bcl-2 gene family (Yu *et al*., 2001). A pro-apoptotic member of the Bcl-2 gene family called Bax increased significantly in a dose-dependent fashion. Meanwhile, Bcl-2, the anti-apoptotic member was declined after As2O3 exposure in chicken testis (Shao *et al.,* 2018). Increased Bax further stimulated Cyt c, thereby activated Caspase-9 up-regulation, and finally generated Caspase-3, which led to apoptosis (Eisenberg-Lerner *et al.,* 2009). This is a pathway to cause apoptosis called the intrinsic

pathway. In chicken testis, after As_2O_3 exposure, intrinsic pathway of apoptosis-related genes' mRNA and protein levels such as Cyt c, p53, Caspase-9 Caspase-3 and Bax were increased as a dose dependent fashion, which meant intrinsic pathway of apoptosis was activated by A_s2O_3 exposure (SHAO *ET AL.*, 2018). In addition, the extrinsic pathway of apoptosis was also activated. In chicken testis, Fas is activated after oxidative stress (SHAO *ET AL.,* 2018), which can be associated with caspase activation, such as Caspase- 8 and Caspase-3 (WAJANT, 2002). Caspase-3 is a key effector molecule for apoptosis execution. Most factors that trigger apoptosis eventually require Caspase-3-mediated signal transduction pathways, leading to apoptosis (RONG *ET AL*., 2008). In chicken testis, As_2O_3 significantly increased Fas, Caspase-8 and Caspase-3 expressions in a dose-dependent fashion, which means extrinsic pathway of apoptosis is activated (SHAO *ET AL.,* 2018). Shao et al. (2018) found that numerous factors factors (Cyt c, p53, Bax, Bcl-2, Caspase-9) controlled intrinsic apoptosis in chicken testis.

CONCLUSION

In summary, during the breeding period of an avian male, the testis undergo many cellular changes related to proliferation and degradation. Proliferation may be regulated by endocrine, paracrine, and autocrine mechanisms driven by hormones, cytokines, and transcriptional regulators. Degradation may be initiated by conditions such as decreasing gonadotropins or testosterone levels or injury, conditions that up-regulate Sertoli cell Fas-ligand, causing apoptosis of germinal cells expressing Fas-receptor. Up-regulation of bcl-2 or related proteins may help to protect cells from apoptosis. In this review, we describe the factors (external and a few internal) are responsible for the ROS production in reproductive organs relative to abnormal spermatogenesis. Free radicals' life-threatening attacks to the body's different organs can cause arterial occlusion and induction of oxidative stress and subsequently causing serious damage to tissues. High production of oxidative tension markers in the testis leads to necrosis or disturb the normal regulatory mechanisms (high cell division, cell contest for oxygen rate, and low oxygen pressure). These conditions lead to destabilized vessels in the testis and excessive production of cholesterol and fatty acids. So, an adverse effect of oxidative stress causes inability to neutralize the antioxidant system, this leads to infertility in males as well as females. Metformin exposure has a negative effect on the germ cell population and also could induce a decrease in testis weight and in spermatid production. Heat stress (HS) causes injuries to the histological structure of pituitary and testis in chicks and seriously

impedes the growth and development of reproductive organs in chicks. Moreover, Histopathological alterations in testis of male chickens given feed containing CSM included increased inter-tubular connective tissue proliferation, presence of necrotic spermatids in the tubular epithelial layer and absence of spermatozoa. The testis of birds fed diets containing JCM were atrophic, with a decrease in the size of seminiferous tubules, which were small and lined with inactive Sertoli cells and rare spermatogonia. Mycotoxins (zearalenone) can cause a decrease in the diameter of the seminiferous tubule and a decrease in catalase activity broiler testis. Pb can cause damage and oxidative stress in the chicken testis via increasing H_2O_2 content; decreasing CAT, TAOC, and SOD activities in the chicken testis. The heavy metal Mn is toxic to testicular cells in birds. The oxidative stress and subsequent DNA damage and cell death induced by Mn are important mechanisms in Mn cytotoxicity for bird testicular cells. Therefore, the mechanism of the effects of Mn on bird health will be the subject of future studies to better understand the toxicity of Mn on bird testicular cells. Exposure to As_2O_3 can cause tissue damage in testis of chickens, which will affect the breeding of the poultry industry and people's economic benefits.We have showed that As_2O_3 exposure led to ultrastructural changes, oxidative stress, apoptosis and autophagy in the testis of chickens. Arsenic-induced oxidative stress can cause the crosstalk between apoptosis and autophagy in testis of chicken.

SUMMARY

Testes are important reproductive organs of chickens, which are related to the production and reproduction of the poultry industry. If the testicles cause poisoning, the quality of sperm in the testes will be affected, eventually leading to sperm dysplasia or deformity, which will affect the success rate of mating. Apoptosis is the major pathway of programmed cell death under developmental and/or stressful conditions and can be triggered via the extrinsic pathway or the intrinsic pathway. In this review, we describe the factors (external and a few internal) are responsible for reactive oxygen species (ROS) production and apoptosis in chicken testis. It was reported that metformin exposure has a negative effect on the germ cell population and also could induce a decrease in testis weight and in spermatid production. Heat stress (HS) causes injuries to the histological structure of pituitary and testis in chicks and seriously impedes the growth and development of reproductive organs in chicks. Moreover, histopathological alterations in testes of male chickens given feed containing cottonseed meal (CSM) included increased inter-tubular

connective tissue proliferation, presence of necrotic spermatids in the tubular epithelial layer and absence of spermatozoa. The testis of birds fed diets containing Jatropha curcas meal (phorbol esters) were atrophic, with a decrease in the size of seminiferous tubules, which were small and lined with inactive sertoli cells and rare spermatogonia. Mycotoxins (zearalenone) can cause a decrease in the diameter of the seminiferous tubule and a decrease in catalase activity broiler testes. Pb can cause damage and oxidative stress in the chicken testes via increasing H2O2 content; decreasing CAT, TAOC, and SOD activities in the chicken testes. The heavy metal Mn is toxic to testicular cells in birds. The oxidative stress and subsequent DNA damage and cell death induced by Mn are important mechanisms in Mn cytotoxicity for bird testicular cells. We have showed that As_2O_3 exposure led to ultrastructural changes, oxidative stress, apoptosis and autophagy in the testes of chickens.

KEYWORDS: testis; apoptosis; reactive oxygen species; heat stress; cytotoxicity

SUMÁRIO

Os testes são importantes órgãos reprodutivos de frangos relacionados à reprodução e produção da indústria avícola. Se os testículos causarem intoxicação, a qualidade do esperma nos testículos será afetada, levando à displasia ou deformidade do esperma, o que afetará a taxa de sucesso do acasalamento. A apoptose é a principal via de morte celular programada em condições de desenvolvimento e/ou estressantes e pode ser desencadeada por via extrínseca ou intrínseca. Nesta revisão, descrevemos os fatores (externos e alguns internos) responsáveis pela produção e apoptose de espécies reativas de oxigênio (ERO) no testículo de frangos. Foi relatado que a exposição à metformina tem um efeito negativo na população de células germinativas e também pode induzir uma diminuição no peso dos testículos e na produção de espermatídeos. O estresse térmico (SH) causa lesões na estrutura histológica da hipófise e testículo em pintos e impede seriamente o crescimento e desenvolvimento de órgãos reprodutivos em pintos. Além disso, as alterações histopatológicas nos testículos de frangos que receberam ração contendo farelo de algodão (CSM) incluíram aumento da proliferação de tecido conjuntivo tubular, presença de espermatídeos necróticos na camada epitelial tubular e ausência de espermatozóides. Os testículos de aves alimentadas com dietas contendo farelo de *Jatropha curcas* (ésteres de forbol) eram atróficos, com uma diminuição no tamanho dos túbulos seminíferos, pequenos e alinhados com células sertoli inativas e espermatogônias raras. As micotoxinas (zearalenona) podem causar uma diminuição no diâmetro do túbulo seminífero e uma diminuição nos

testículos de atividade da catalase. O Pb pode causar danos e estresse oxidativo nos testículos de framgps através do aumento do conteúdo de H_2O_2; diminuindo as atividades de CAT, TAOC e SOD nos testículos de frangos. O metal pesado Mn é tóxico para as células testiculares em aves. O estresse oxidativo e o subsequente dano ao DNA e a morte celular induzidos pelo Mn são mecanismos importantes na citotoxicidade do Mn para células testiculares de aves. Mostramos que a exposição ao As_2O_3 levou a alterações ultraestruturais, estresse oxidativo, apoptose e autofagia nos testículos de frangos.

Palavras-chave: testes; apoptose; espécies reativas de oxigênio; estresse térmico; citotoxicidade

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BIBLIOGRAPHY

Abdennadher, A; F. Ramirez; M. S, Romdhane: X. Ruiz; L. Jover & C. Sanpera. 2010. Biomonitoring of coastal areas in Tunisia: stable isotope and trace element analysis in the Yellow-legged Gull. *Marinepollution Bull. 60:* 440-7.

Abdul, K. S.; S. S. E. P. Jayasinghe; C. Chandana, C. Jayasumana & M. De Silva. 2015. Arsenic and human health effects: A review. *Chemosphere. 40*: 238–245.

Allen, N. K.; C. J. Mirocha; G. Weaver; S. Aakhus-Allen & F. E. Bates. 1981. Efects of dietary zearalenone on finishing broiler chickens and young turkey poults. *Poult. Sci. 60:* 124–131.

Baldini, M.; S. Raranciuc & M. Vischi. 2012. Toxicity of Jatropha curcas L. as a biofuel crop and options for management. *CAB Reviews 7* (42):1-13.

Barros I. C. R.; M. A. M. Rodrigues; F. M. Nunes; M. C. M. Kasuya; J. M. R. da Luz; A. Alves; L. M. M. Ferreira; V. Pinheiro & J. L. Mourão. 2015. *The Effect of* Jatropha curcas *Seed Meal on Growth Performance and Internal Organs Development and Lesions in Broiler Chickens. Rev. bras. Cienc. Avic. 17* (no.spe), Campinas Oct./Dec.

Basu, A., & S. Haldar, 1998. The relationship between BcI2, Bax and p53: Consequences for cell cycle progression and cell death. M*ol. Hum. Reprod. 4:* 1099–1109.

Berglund A. M; P. K. Ingvarsson; H. Danielsson, N. E. Nyholm. 2010. Lead exposure and biological effects in pied flycatchers (*Ficedula*

hypoleuca) before and after the closure of a lead mine in northern Sweden. *Environmental pollution., 158:* 1368-1375.

Bongaerts, G. P. 2008. What of apoptosis is important: the decay process or the causative origin? *Med. Hypotheses. 70*: 482–487.

Brandon, E. F., P. J. Janssen, & L. de Wit-Bos. 2014. Arsenic: bioaccessibility from seaweed and rice, dietary exposure calculations and risk assessment. Food Addit. Contam. Part A *Chem. Anal. Control Expo. Risk Assess. 31*: 1993–2003.

Chandra, S.V., R. Ara; N. Nagar & P. K . Seth. 1973. *Steril.,* 857–862.

Chen Z., Zhang J. R., Zhou Y. Liang W., C. & Jiang Y. Y. 2015. Effect of heat stress on the pituitary and testicular development of Wenchang chicks. A*rch. Anim. Breed., 58:* 373–378.

Chen, Y. C., S. Y. Lin-Shiau, & J. K. Lin. 1998. Involvement of reactive oxygen species and caspase 3 activation in arsenite-induced apoptosis. *J. Cell. Physiol., 177*:324–33

Chen, Z.; J. Xie; B. Wang & J. Tang. 2014. Effect of aminobutyric acid on digestive enzymes, absorption function, and immune function of intestinal mucosa in heat-stressed chicken. *Poultry Sci., 93*: 2490–2500.

Çiftçi M; U. G. Pimpek; M. A. Azman;, Y. H. Çerçi & F. Tonbak. 2013. The effects of dietary rosemary (Rosmarinus officinalis L.) oil supplementation on performance, carcass traits and some blood parameters of Japanese quail under heat stressed condition. *Kafkas Univ. Vet. Fak. Derg. 9:* 595-599.

Cortinovis, C.; F. Pizzo; L. J. Spicer & F. Caloni. 2013. Fusarium mycotoxins: E_ects on reproductive function in domestic animals—A review. *Theriogenology, 80:* 557–564.

Darbandi, M.; S. Darbandi; A. Agarwal; P. Sengupta; D. Durairajanayagam; R. Henkel & M. R. Sadeghi. 2018. Reactive oxygen species and male reproductive hormones. *Reproductive Biology and Endocrinology:16* (1): 87.

Dauwe, T., E. Janssens; B. Kempenaers; M. Eens. 2004. The effect of heavy metal exposure on egg size, eggshell thickness and the number of spermatozoa in blue tit Parus caeruleus eggs. *Environ. Pollut. 82:* 301–310.

De Francisco N.; J. D. Ruiz Troya & E. I. Aguera. 2003. Lead and lead toxicity in domestic and free living birds. *Avian pathol. Journ. WVPA., 32:* 3-13.

Dkhil M.A.; A. E. Moneim & S. Al-Quraishy. 2016. *Indigofera oblongifolia* ameliorates lead acetate-induced testicular oxidative

damage and apoptosis in a rat model. *Biolog. Trace Element Res. 173*: 354-361.

Durairajanayagam D.; R. K. Sharma; S. S. du Plessis; A. Agarwal. 2014. *Testicular heat stress and sperm quality. In: du Plessis SS, Agarwal A, Sabanegh Jr. ES, editors. Male Infertility: A Complete Guide to Lifestyle and Environmental Factors.* Springer Science and Business Media. New York, USA. Pp 105-125.

Ebeid T. A. 2012. Vitamin E and organic selenium enhances the antioxidative status and quality of chicken semen under high ambient temperature. *Br. Poult. Sci.* 53: 708-14.

Eichler, T.; Q. Ma; C. Kelly; J. Mishra; S. Parikh; R. F. Ransom: P. Devarajan: .... Smover, A. Eisenberg-Lerne; S. Bialik; H. U. Simon, & A. Kimchi. 2009. Life and death partners: apoptosis, autophagy and the cross-talk between them. *Cell Death Differ. 16*: 966–975.

El Mchichi, B.; A. Hadji; A. Vazquez; G. Leca. 2007. P38 MAPK and MSK1 mediate caspase-8 activation in manganese-induced mitochondria-dependent cell death. *Cell Death Differ. 14* (10): 1826–1836

El-Badwi S. M.; S. E. Adam; H. J. Hapke. 1995. Comparative toxicity of *Ricinus communis* and *Jatropha curcas* in brown hisex chicks. *Deutsche Tierärztliche Wochenschrift. 102* (2): 75-77.

El-Badwi, S. M.; A.E. Adam. 1992. Toxic effects of low levels of dietary Jatropha curcasseed on brown hisex chicks*. Veterinary and Human Toxicology, 34* (2): 112-115.

European Food Safety Authority (EFSA). 2015. *Opinion of the Scientific Panel on Contaminants in Food Chain on a Request from the Commission Related to Fumonisins as undesirable substances in animal feed. EFSA, Parma, Italy*.

Ewuola, E. O, & G. N. 2010a. Egbunike Efects of dietary fumonisin B1 on the onset of puberty, semen quality, fertility rates and testicular morphology in male rabbits. *Reproduction, 139:* 439–445.

Ewuola, E. O. & G. N. Egbunike. 2010b. Gonadal and extra-gonadal sperm reserves and sperm production of pubertal rabbits fed dietary fumonisin B1. *Anim. Reprod. Sci. 119:* 282–286.

Ezzat W.; M. S. Shoeib; S. M. M. Mousa; A. M. A. Bealish & Z. A. Ibrahiem. 2011. Impact of betaine, vitamin C and folic acid supplementations to the diet on productive and reproductive performance of Matrouh poultry strain under Egyptian summer condition. *Egypt Poult. Sci. 31*: 521-37.

Faure M., E. Guibert, S. Alves; B. Pain; C. Ramé; J. Dupont; J. P. Brillard & P. Froment. 2016. The insulin sensitiser metformin

regulates chicken Sertoli and germ cell populations. *Reproduction.151* (5): 527-38.

Feng, Q.; P. Li; C. Salamanca; D. Huntsman; P. C. Leung & N. Auersperg. 2005. Caspase-1alpha is down-regulated in human ovarian cancer cells and the overexpression of caspase-1alpha induces apoptosis. *Cancer Res. 65:* 8591-8596.

Gafvels, M.; J. Wang; A. Bergh; Damber; J. E. & G. Selstan. 1984. Toxic effects of the antifertility agent gossypol in male rats. *Toxicology, 32*: 325-333.

Gao, S.; X. Duan; X. Wang; D. Dong; D. Liu; X. Li; G. Sun & B. Li. 2013. *Curcumin attenuates* arsenic-induced hepatic injuries and oxidative stress in experimental mice through activation of Nrf2 pathway, promotion of arsenic methylation and urinary excretion. *Food Chem. Toxicol. 59:* 739–747.

Gil-Sanchez J.M.; S. Molleda; J. A. Sanchez-Zapata: J. Bautista; I. Navas; R. Godinho. 2018. From sport hunting to breeding success: Patterns of lead ammunition ingestion and its effects on an endangered raptor. *Scien.Tot. Environ. 613* (614): 483-491.

Gong, R. M.; D. M. Zhang; K. D. Zhong; M. Feng; X. Y. Liu. 2008. Determination of trace copper in water samples by flame atomic absorption spectrometry after preconcentration on a phosphoric acid functionalized cotton chelator. *J. Serb. Chem. 73 (*2), 249–258.

Guerre, P. 2016. Worldwide Mycotoxins Exposure in Pig and Poultry Feed Formulations. *Toxins 8:* 350.

Han Y, C. Li; M. Su; Z. Wang; N. D. S. Jiang. 2017. Antagonistic effects of selenium on lead-induced autophagy by influencing mitochondrial dynamics in the spleen of chickens. *Oncotarget. 8*: 33725-33735.

Hanahan D. & R. A. Weinberg. 2000. The hallmarks of cancer. *Cell 100:* 57-70.

Hasanein P., F. Fazeli; M. Parviz & M. Roghani. 2018. Ferulic acid prevents lead-induced testicular oxidative stress and suppressed spermatogenesis in rats. *Andrologia:* 50 (4).

Heydari, M. J.; S. Mohammadzadeh; A. Kheradmand & M. Alirezaei. 2015. Effect of dietary Satureja khuzistanica powder on semen characteristics and thiobarbituric acid reactive substances concentration in testicular tissue of Iranian native breeder rooster. *IJVR,* Series 52, *16,* (3): 255-260.

Hirata, Y. 2002. Manganese-induced apoptosis in PC12 cells. *Neurotoxicol. Teratol. 24* (5), 639–653.

Huang, C.; C. Lai; P. Xu; G. Zeng; D. Huang; J. Zhang .... . 2017. Lead-induced oxidative stress and antioxidant response provide insight into

the tolerance of *Phanerochaete chrysosporium* to lead exposure. *Chemosphere. 187*: 70-77.

Huang H, Y. Wang; Y. An; W. Jiao;Y. Xu; Q. Han & X. Teng. 2019. Selenium alleviates oxidative stress and autophagy in lead-treated chicken testis. *Theriogenology, 131*: 146-152.

Huang, H,; Y. Wang; Y. An; Y. Tian; S. Li; X. Teng. 2017. Selenium for the mitigation of toxicity induced by lead in chicken testis through regulating mRNA expressions of HSPs and selenoproteins. *Environ. Science Pollution Research International. 24*: 14312-14321.

Janssen, D. L.; T. Dauwe; R. Pinxten; L. Bervoets; R. Blust & M Eens, 2003. Effects of heavy metal exposure on the condition and health of nestling of the great tit (*Parus major*), a small songbirds species. *Environ. Pollut. 126:* 267–274.

Jomova, K.; D. Vondrakova; M. Lawson & M. Valko. 2010. Metals, oxidative stress and neurodegenerative disorders. *Mol. Cell. Biochem. 345:* 91–104.

Kalla, N.R.; V. K. Chadha & J. Foo. 1990. Effect of gossypol on domestic fowl, gallus domesticus. *Acta Eur. Fertil., 21*: 21-24.

Khalil, S. R.; W. M. Elhady; Y. H. A. Elewa; N. E. Abd El-Hameed & S. A. Ali. 2018. Possible role of Arthrospira platensis in reversing oxidative stress-mediated liver damage in rats exposed to lead. *Biomed. & Pharmacother-Biomed. & Pharmacot., 97:* 1259-1268.

Khan, R. U. 2011. Antioxidants and poultry semen quality. *World's Poult. Sci. J., 67*: 297-308.

Kim, Y. R.; K. M. Kim; N. J; Yoo & S.H. Lee. 2009. Mutational analysis of CASP1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 14 genes in gastrointestinal stromal tumors. *Hum. Pathol. 40*: 868-871.

Kosicki, R.; A. Bajet-Kosicka; J. Grajewski; M. Twaruzÿek. 2016. Multiannual mycotoxin survey in feed materials and feedingstu. *Anim. Feed Sci. Technol. 215:* 165–180.

Kumar, S. 1999. Mechanisms mediating caspase activation in cell death. *Cell Death Differ. 6*: 1060-1066.

Lee, J.; J. H. Richburg; S. C. Younkin & K. Boekelheide, 1997. The Fas system is a key regulator of germ cell apoptosis in the testis. *Endocrinology, 138:* 2081–2088.

Leo, C. P.; S. Y. Hsu; E. A. McGee; M. Salanova & A. J. Hsueh, 1998. DEFT, a novel death effector domain-containing molecule predominantly expressed in testicular germ cells. *Endocrinology, 139*: 4839–4848.

Lessene, G.; P. E. Czabotar & P. M. Colman. 2008. BCL-2 family antagonists for cancer therapy. *Nat. Rev. Drug Discov., 7*: 989-1000.

Levine, B. & G. Kroemer. 2008. Autophagy in the pathogenesis of disease. *Cell 132*: 27-42.

Li, Y. Z. & Y. Q. Cui. 2013. Effects of heat stress on reproductive performance, blood reproductive hormone concentrations and gene mRNA expression in different varieties of chicken. *J. China Agri. Univ., 18*, 134–141 (in Chinese).

Li, Y.; T. Cai; Y. Zhang,; S. Lv; Y. Wang; L.Ye. 2010. Alpha-synuclein overexpression during manganese-induced apoptosis in SH-SY5Y neuroblastoma cells. *Brain Res. Bull. 81* (4–5): 428–433.

Lin, Y. C.; T. Dietrick, Y. Rikihisa & W. L Beane. 1988. Antifertility effect of gossypol in male Japanese quail. *Life Sci., 42:* 2283-2292.

Liu, X. F.; L. M. Zhang; H. N. Guan; Z. W. Zhang & S. W. Xu. 2013. Effects of oxidative stress on apoptosis in manganese-induced testicular toxicity in cocks. *Food Chem. Toxicol., 60*: 168-76.

Liu, X.; Z. Li; V. Han; Z. Zhang & S. Xu. 2012. Effects of dietary manganese on Cu, Fe, Zn, Ca, IL-1b, and IL-2 changes of immune organs in cocks. *Biol. Trace Elem. Res. 148* (3): 336–344.

Mabrouk A. & H. Ben Cheikh 2015. Thymoquinone supplementation reverses lead-induced oxidative stress in adult rat testis. *General Physiol. Biophys., 34*: 65-72.

Maheshwari, A. & M. M. Misro. 2009. Aggarwal A, Sharma RK, Nandan D. Pathways involved in testicular germ cell apoptosis induced by H2O2 in vitro. *FEBS J. 276:* 870-881.

Mahmood, F; K. Muhammad Zargham; A. Khan. 2011. Lysine Induced Modulation of Toxico-Pathological Effects of Cottonseed Meal in Broiler Breeder Males. *Pakistan Journal of Zoology*; Lahore, *43* (2).

Makkar, H. P. S.; K. Becker; F. Sporer; M. Wink. 1997. Studies on Nutritive Potential and Toxic Constituents of Different Provenances of *Jatropha curcas. Journ. Agricult. Food Chemist. 45* (8): 3512-3557.

Makkar, H. P. S. & K; Becker. 1998. Jatropha curcas *toxicity: Identification of toxic principle (s). In*: Garland T, Barr AC, editors. *Toxic plants and other natural toxicants*. New York: CAB International. Pp. 554-558.

Makkar, H. P. S.; G. Francis; K. Becker. 2008. Protein concentrate from Jatropha curcas screw-pressed seed cake and toxic and antinutritional factors in protein concentrate. *Journ. Science Food Agric., 88* (9):1542-1548.

Martinez, V. D.; E. A. Vucic, D. D. Becker-Santos, L. Gil, & W. L. Lam. 2011. Arsenic exposure and the induction of human cancers. *J. Toxicol. 2011*: 431287.

Mathur, P. P. & S. C. D'Cruz. 2011. The effect of environmental contaminants on testicular function. *Asian Jour. Androl.*, *13* (4): 585-591.

McClure, R. F.; C. J. Heppelmann & C. V. Paya. 1999. Constitutive Fas ligand gene transcription in Sertoli cells is regulated by *Sp1. J. Biol. Chem. 274*: 7756–7762.

McDaniel C. D., J. E. Hood & H. M. Parker. 2004. An attempt at alleviating heat stress infertility in male broiler breeder chickens with dietary ascorbic acid. *Int. J. Poult. Sci., 3*: 593-602.

Murth, R.C.; R. S. Srivastava; S. K. Gupta & S. V. Chandra. 1980. Manganese induced testicular changes in monkeys. *Exp. Path. 18:* 224–224.

Murugesan, G. R.; D. R. Ledoux; K. Naehrer; F. Berthiller; T. J. Applegate; B, Grenier; T. D. Phillips & G. Schatzmayr. 2015. Prevalence and e_ects of mycotoxins on poultry health and performance, and recent development in mycotoxin counteracting strategies. *Poult. Sci. 94*: 1298–1315.

Nagalakshmi, D; S. V. Rama; A. K. Panda & V. R. B. Sastry. 2007. Cottonseed Meal in Poultry Diets: A Review. *J. Poult. Sci., 44:* 119-134.

Nardone, A.; B. Ronchi; N. Lacetera; M; S. Ranieri & U. Bernabucci. 2010. Effects of climate changes on animal production and sustainability of livestock systems. *Livestock Sci., 130:* 57-69.

Nicholson D.W. 1999. Caspase structure, proteolytic substrates, and function during apoptotic cell death. *Cell Death Differ 6*: 1028-1042.

Ommati, M. M.; M. J. Zamiri; A. Akhlaghi; H. Atashi; M; R; Jafarzadeh; M. R. Rezvani & F. Saemi. 2013. Seminal characteristics, sperm fatty acids, and blood biochemical attributes in breeder roosters orally administered with sage (*Salvia officinalis*) extract. *Anim. Product. Sci., 53*: 548-554.

Orhan. C.; F. Akdemir; N. Sahin; M. Tuzcu; J. R. Komorowski; A. Hayýrlý .... . 2012. Chromium histidinate protects against heat stress by modulating the expression of hepatic nuclear transcription factors in quail. *Br. Poult. Sci. 53*: 828-35.

Oubrahim, H., Chock, P.B., Stadtman, E.R., 2002. Manganese (II) induces apoptotic cell death in NIH3T3 cells via a caspase-12-dependent patheway. J. Biol. Chem. 277 (23), 20135–20138.

Pentikainen, V.; K. Erkkila, ; L. Dunkel. 1999. Fas regulates germ cell apoptosis in the human testis *in vitro*. *Am. J. Physiol. 276*: 310–316.

Pinotti, L.; M. Ottoboni; C. Giromini; V. Dell'Orto & F. Cheli. 2016. Mycotoxin contamination in the EU feed supply chain: a focus on cereal byproducts. *Toxins, 8:* 45.

Ponnapakkam, T.P.; G. H. Sam; M. B. Iszard. 2003. Histopathological changes in the testis of the Sprague Dawley rat following orally administered manganese. *Bull. Environ. Contam. Toxicol. 71* (6): 1151.

Rakshit K. D. & S. Bhagya. 2008. Biochemical and nutritional evaluation of Jatropha protein isolate prepared by steam injection heating for reduction of toxic and antinutritional factors. *Jour. Science Food Agric. 88* (5): 911-919.

Rasooli, A.; M. T. Jalali; M. Nouri; B. F. Mohammadian & B. F. Barati. 2010. Effects 1 of chronic heat stress on testicular structures, serum testosterone and cortisol concentrations in developing lambs. *Anim. Reprod., Sci. 117*: 55-9.

Rehman, S., Z. Usman; S. Rehman; M. AlDraihem; N. Rehman; I. Rehman & G. Ahmad. 2018. Endocrine disrupting chemicals and impact on male reproductive health. *Translat. Androl. Urol. 7* (3): 490-503.

Richburg, J. H. & K. Boekelheide. 1996. Mono-(2-ethylhexyl) phthalate rapidly alters both sertoli cell vimentin flaments and germ cell apoptosis in young rat testis, *Toxicol. Appl. Pharmcol., 137:* 42–50.

Rong, J.; W. Chang; L. Lv & J. Chen. 2008. Study on the roles of nuclear factor-kappaB, p53 and Bcl-2 gene in lead acetate induced apoptosis in PC12 cells. *Wei Sheng Yan Jiu. 37*: 262–268.

Ryan, J. R.; F. H. Kratzer; C. R. Grace & P. Vohra. 1986. Glandless cottonseed meal for laying and breeding hens and broiler chicks. *Poult. Sci., 65*: 949-955.

Sahin, N; M. Onderci; K. Sahin; O. Kucuk. 2008. Supplementation 1 with organic or inorganic selenium in heat-distressed quail. *Biol. Trace Elem. Res. 122*: 229-37.

Sakellarides, T. M.: I. K. Konstantinou; D. G. Hela; D. Lambropoulou; A. Dimou & T. A. Albanis. 2006. Accumulation profiles of persistent organochlorines in liver and fat tissues of various waterbird species from Greece. C*hemosphere 63*: 1392–1409.

Saksena, S.K.; R. Salmonsen; I. F. Lau; & M. C. Chang. 1981. Gossypol: Its toxicological and endocrinological effects in male rabbits. *Contraception, 24*: 203-214.

Samuel, S.; R. Kathirvel; T. Jayavelu & P. Chinnakkannu. 2005. Protein oxidative damage in arsenic induced rat brain: influence of DL-alpha-lipoic acid. T*oxicol. Lett. 155*: 27–34.

Schwalm, A.; m. Gauly; g. Erhardt & m. Bergmann. 2007. Changes in testicular histology and sperm quality in llamas (*Lama glama*), following exposure to high ambient temperature. *Theriogenology 67*: 1316-1323.

Shao, Y. Z.; H. J. Zhao; Y. Wang; J. J. Liu; J. L. Li; L. Y. Luo & M. W. Xing. 2018. The apoptosis in arsenic-induced oxidative stress is associated with autophagy in the testis tissues of chicken. *Poult Sci., 97* (9): 3248-3257.

Sinha Hikim & A. P.; R. S. Swerdloff . 1999. Hormonal and genetic control of germ cell apoptosis in the testis. *Rev. Reprod. 4*: 38-47.

Siva Kumar, T. & P. Neeraja. 2019. *Factors Associated With Oxidative Stress in the Testes and the Mitigating Role of Antioxidants: A Review. Intern. Jour. Recent Innov. Medicine and Clinical Research 1*(1): 6-12.

Slee E.A.; M. T. Harte & R. M. Kluck. 1999. Ordering the cytochrome c-initiated caspase cascade: hierarchical activation of caspases-2, -3, -6, -7, -8, and -10 in a caspase-9-dependent manner. *J. Cell. Biol. 144:* 281-292.

Smith, K. J. 1970. Practical significance of gossypol in feed formulation. *JAOCS, 47*: 448-450.

Spencer, A. 1999. Whole blood manganese levels in pregnancy and the neonate. *Nutrition 15:* 731–734.

Sun, X.; S. Li; Y. He; H. Zhao; Y. Wang; X. Zeng & M. Xing. 2017. Arsenic-induced testicular toxicity in *Gallus gallus*: Expressions of inflammatory cytokines and heat shock proteins. ....

Surai P. F.; N. Fujihara; B. K. Speake; J. P. Brillard; G. J. Wishart & N. H C. Sparks. 2001. Polyunsaturated fatty acids, lipid peroxidation and antioxidant protection in avian semen. *Asian-Aust. J. Anim. Sci. 14:* 1024-50.

Svechnikov K; C. Spatafora; I. Svechnikova; C. Tringali C & O. Söder. 2009 Effects of resveratrol analogs on steroidogenesis and mitochondrial function in rat Leydig cells in vitro. *Journ. Appl. Toxic. 29* 673–680.

Takeda, A. 2003. Manganese action in brain function. *Brain Res. Rev. 41:* 79–87.

Tartarin, P; E. Guibert; A. Touré; C. Ouiste; J. Leclerc; N. Sanz; S. Brière; J. L. Dacheux: B Delaleu; J. R. McNeilly. ... 2012a. Inactivation of AMPKá1 induces asthenozoospermia and alters spermatozoa morphology. *Endocrinology 153:* 3468–3481.

Terim Kapakýn, K. A.; H. Imýk; R. Gumus; S. Kapakýn & Y. S. Saglam 2013. Effect of vit E on secretion of HSP-70 in testes of broilers exposed to heat stress. *Kafkas Univ. Vet. Fak. Derg., 19:* 305-10.

Thurston R. J. & N. Korn. 2000. Spermiogenesis in commercial poultry species: anatomy and control. *Poultry Science, Volume 79:* 1650–1668.

Tonbak, F. & M. Çiftçi. 2012. Effects of cinnamon oil (Cinnamomum zeylanicum L.) supplemented to ration on growth performance and carcass characteristics in heat stressed Japanese quails (in Turkish). *FÜ Sað Bil Vet Derg 26*: 157-64.

Türk G.; U. G. Þimþek; A. O. Çeribaþý; S. Çeribaþý; Þ. Özer Kaya; M. Güvenç; M. Çiftçi; M. Sönmez; A. Yüce; A. Bayrakdar; M. Yaman & F. Tonbak. 2015. Effect of cinnamon (*Cinnamomum zeylanicum*) bark oil on heat stress-induced changes in sperm production, testicular lipid peroxidation, testicular apoptosis, and androgenic receptor density in developing Japanese quails. *Theriogenology. 84* (3): 365-76.

Vallverdú-Coll, N; F. Mougeot; M; E. Ortiz-Santaliestra; C. Castaño; J. Santiago-Moreno; R. Mateo. 2016. Effects of lead exposure on sperm quality and reproductive success in an avian model. . *Environ Sci Technol. 50*: 12484-12492.

Vilagrassa, X.; C. Mezquita & J. Mezquita. 1997. Differential expression of bcl-2 and bcl-x during chicken spermatogenesis. *Mol. Reprod. Dev. 47:* 26–29.

Wajant, H. 2002. The Fas signaling pathway: more than a paradigm. *Science. 296*: 1635–1636.

Wirth, J. J.; M. G. Rossano; D. C. Daly; N. Paneth; E. Puscheck; R. C. Potter & M. P. Diamond. 2007. Ambient manganese exposure is negatively associated with human sperm motility and concentration. *Epidemiology 18* (2): 270–273.

Xie, J. 2013. Protective effect of aminobutyric acid on antioxidation function in intestinal mucosa of Wenchang chicken induced by heat stress, *J. Anim. Plant. Sci., 23*: 1634–1641.

Xie, L.; Z. Wang; C. Li; K. Yang & Y. Liang. 2017. Protective effect of nicotinamide adenine dinucleotide (NAD+) against spinal cord ischemia-reperfusion injury via reducing oxidative stress-induced neuronal apoptosis. *J. Clin. Neurosci. 36*: 114– 119.

Xu C; B. Bailly-Maitre & J. C. Reed. 2005. Endoplasmic reticulum stress: cell life and death decisions. *J. Clin. Invest. 115*: 2656-2664.

Yang F, J. Liao; R. Pei ; W. Yu; Q. Han; Y. Li. 2018. Autophagy attenuates copper-induced mitochondrial dysfunction by regulating oxidative stress in chicken hepatocytes. *Chemosphere.* (204): 36-43.

Yang, J.; Y. Zhang; S. Hamid; J. Cai; Q. Liu; H. Li; R. Zhao; H. Wang; S. Xu & Z. Zhang. 2017. Interplay between autophagy and apoptosis in selenium deficient cardiomyocytes in chicken. *J. Inorg. Biochem. 170:* 17–25.

Yin Y; K. L. Hawkins; W. C. Dewolf & A. Morgentaler. 1997. Heat stress causes testicular germ cell apoptosis in adult mice. *J. Androl. 18:* 159-65.

Yip K.W. & J. C. Reed. 2008. Bcl-2 family proteins and cancer. *Oncogene 27*: 6398-6406.

Yu, X., H. Kubota, R. Wang, J. Saegusa, Y. Ogawa, G. Ichihara, Y. Takeuchi, & N. Hisanaga. 2001. Involvement of Bcl-2 family genes and Fas signaling system in primary and secondary male germ cell apoptosis induced by 2-bromopropane in rat. *Toxicol. Appl. Pharmacol. 174*: 35–48.

Zachariasova, M.; Z. Dzuman; Z. Veprikova; K. Hajkova; M. Jiru; M. Vaclavikova; A. Zachariasova; M. Pospichalova; M. Florian; J. Hajslova. 2014. Occurrence of multiple mycotoxins in European feedingstu_s, assessment of dietary intake by farm animals. *Anim. Feed Sci. Technol. 193*: 124–140.

Zang M; S. Xu; K. A. Maitland-Toolan; A. Zuccollo; X. Hou; B. Jiang, M. Wierzbicki, T. J. Verbeuren & R. A. Cohen. 2006. Polyphenols stimulate AMP-activated protein kinase, lower lipids, and inhibit accelerated atherosclerosis in diabetic LDL receptor-deficient mice. *Diabetes 55*: 2180–2191.

Zhang J.; t. Cai; f. Zhao; T. Yao; Y. Chen; x. Liu... . 2012. The role of alpha-synuclein and tau hyperphosphorylation-mediated autophagy and apoptosis in lead-induced learning and memory injury. *Int. Jour. Biol. Scien. 8*: 935-44.

Zhang, D., X. He; S. Huang & Y. Li. 2002. Toxicity of manganese exposure on the postnatal development of brain in mice. *Wei Sheng Yan Jiu 31*: 73–75 (in Chinese).

Zhang, D., X. He; W. Zhang & J. Tan, 1998. Effect of manganese on: 237–240 (in Chinese).

Zhao, H.; Y. He; S. Li; X. Sun; Y. Wang, Y. Shao, Z. Hou, & M. Xing. 2017a. Subchronic arsenism-induced oxidative stress and inflammation contribute to apoptosis through mitochondrial and death receptor dependent pathways in chicken immune organs. *Oncotarget. 8:* 40327–40344.

Zhao, P.; Y. Guo; W. Zhang; H. Chai; H. Xing; & M. Xing. 2017b. Neurotoxicity induced by arsenic in *Gallus gallus*: Regulation of oxidative stress and heat shock protein response. *Chemosphere. 166:* 238–245.

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