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Pesticide residues in relation to endocrine profile and semen quality in crossbred breeding bulls: A review

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Abstract
Animal and human exposure to pesticides is primarily through food and water ingestion, inhalation, and absorption through the skin. The primary route of exposure in dairy animals is feed and forage. As some of them are highly regulated and some have been discontinued, the likelihood of exposure to OCPs is dwindling. OCPs have been the major agricultural pesticide being used in the past, owing to their high insecticidal activity, low toxicity in mammals, low cost, and long-lasting pest protection. Pesticides act as endocrine disruptors and cause enormous disturbances in steroidogenesis, spermatogenesis, and sexual behavioural display. Due to inadvertent use of potentially hazardous pesticides, dairy animals are highly vulnerable to soil, food, water, and animal-derived oil or fat products incorporated in processed feed.

Keywords: Indian mustard, path coefficient analysis

Introduction

Indian economic contribution and status of artificial insemination

The Indian agricultural economy is highly dependent on various pesticides in order to have a better crop yield and this has ultimately affected our food chains since excessive use of pesticides have markedly affected every tier of our food chains, be it humans or be it animals. Pesticides contaminate the environment, soil and as a result the agricultural products and animals are exposed to the pesticides through feed, water and air. In India, among different states maximum consumption of pesticides in the year 1999-2000 was in Uttar Pradesh (7459 MT) followed by Punjab (6972 MT), Haryana (5025 MT), Andhra Pradesh (4054 MT), Gujarat (3646 MT) (Source: Pesticide Information, Volume XXVIII, No. 3, October-December 2002). The most commonly used pesticides in India include monocrotophos (10,700 MT), acephate (6,400 MT), endosulfan (5,600 MT) and chlorpyrifos (5,000 MT) (Source: Pesticide Information, Volume XXVIII, No. 3, October- December 2002). The state of Punjab follows two crop cycles alternating between *kharif* and *rabi* crops. Punjab state has 15 percent geographical area of the country and produces 22 percent wheat, 10 percent rice and 8 percent cotton of the country (Bajinder 2008). In a previous survey with regard to pesticide use on grain crops and fodder crops revealed that the organophosphorus pesticides (OPPs) like chlorpyriphos, monocrotophos, profenophos, ethion, triazophos, acephate, dichlorovos and quinalphos were more commonly used by the farmers of low pesticide usage area than in high pesticide usage area of punjab (Upasana 2012) [78].

Pesticides: The endocrine disrupters

A pesticide is defined as "any substance or combination of substances used to prevent or eradicate unwanted insects, including disease vectors in humans and animals, weeds, mushrooms, to enhance food production and to help process, store, transport or market food and agricultural commodities" (Abdollahi *et al.* 2004; Clementi *et al.* 2008; Shadnia *et al.* 2005) [1, 27, 58]. Pesticides act as endocrine disrupting chemical substances (EDCs). The U.S. Environmental Protection Agency (EPA) defines the EDC as "an exogenous agent potentially capable of synthesizing, secreting, transporting, binding, acting or eliminating the natural hormones responsible for maintaining the body's homeostasis, reproductive and developmental processes" (Sifakis *et al.* 2011) [64].

Animals are often exposed to a toxicant mixture, and not to a single agent. The effects of that mixture's ingredients may be synergistic or antagonistic (Sifakis *et al.* 2011) [64]. As a major class of broad-spectrum organic insecticides, synthetic pyrethroids are widely used in agricultural, domestic, forestry, horticulture, and veterinary applications worldwide due to their low toxic effects on mammals and birds and limited soil persistence.

In addition, organochlorine and organophosphate insecticides have been banned because of their highly toxic and environmentally friendly persistence (Bradberry *et al.* 2005; Singh and Singh, 2008; Shi *et al.* 2011; Ansari *et al.* 2011; Zeng *et al.* 2015) [11, 5, 62, 66, 82]. Parasiticides and antibiotics are two of the most important groups and are used in animal treatment as such fairly often. (Abdulhakeem *et al.* 2006) [2].

The male sexual functions are very sensitive to pharmacological agents. Male reproductive function is known to be highly sensitive to many chemicals and physical agents generated by industrial or agricultural activities (Bonde 1996; Spira and Multigner 1998; Favareto *et al.* 2011) [10, 72, 32]. Numerous investigations were carried out to detect the effect of drugs on male sexual functions, different chemical classes of Pesticides and solvents have been demonstrated to be male reproductive toxicants in animal models (Sundaram and Witorsch 1995) [74]. Research findings have shown that both synthetic and natural drugs have significant effects on the male reproductive system, especially the domestic and human spermatozoa (Etta *et al.* 2009) [29].

The impact of pollutants on the male reproductive function remains to be fully appreciated and assessed, based on current knowledge (Cillo *et al.* 2007) [16]. Environmental pollutants were found in human semen (Dallinga *et al.* 2002) [21] as well as in farm animals (Kamarianos *et al.* 2003a) [43]. Density, motility and morphology of spermatozoa are reported to be negatively affected by the environmental pollutants (Abell *et al.* 2000) [3]. Heavy metals and agro-pesticides (both organochlorine and organophosphorous) have been shown to damage testicular germ and somatic cells, with genotoxic and teratogenic effects (Eduardo *et al.* 2001; Bonde *et al.* 2002; Thompson and Bannigan 2008) [26, 9, 76]. The spermatozoon, a highly differentiated cell, has a condensed nucleus that is metabolically inactive, and a relatively restricted cytoplasmic compartment. Due to the exceptionally large surface-to-volume ratio of the spermatozoa (more than 50:1), they are more prone to negative effects of pesticides. Therefore, due to adverse effects on cell function of the spermatozoa, pesticide may affect fertilization outcome. If both males and females were to be affected by the toxicants, the spermatozoa could well serve as a readily accessible pollution level index (Nelson 1990) [48]. However, *in vitro* results were conflicting regarding the effect of pollutants on motility, the potential for mitochondrial membrane and the acrosome reaction of spermatozoa (Pflieger-Bruss and Schill 2000) [51].

Scientists have increasingly reported in recent years that certain pollutants can play an important role in the environment, contributing at least in some cases to underlying causes of fertility problems in farm animals (Kamarianos *et al.* 2003b; Campagna *et al.* 2009) [44, 13]. Cadmium induces membrane impairment, decreases motility and decreases the rate of acrosome reactions that result in male infertility (Arabi and Mohammadpour 2006) [6]. The animals exposed to lead had reduced levels of serum testosterone (Hsu *et al.* 1998) [40] and a premature breakdown of acrosome (Johansson 1989) [41]. Chlorpyrifos may modulate *in vivo* phosphorylation of various nuclear protamines of chromatin of rat spermatozoa leading to adverse reproductive effects (Kadavil *et al.* 2001) [42]. Endosulfan impaired testicular functions by altering the spermatogenic enzyme activities (Sinha *et al.* 1995) [67]. Male reproductive functions are interfered by environmental contaminants such as halogenated hydrocarbons and heavy metals (De Celis *et al.* 1996) [24].

Organochlorine pesticides, for example, can affect reproductive function through their interaction with androgen and estrogen receptors (Colborn *et al.* 1993; Gray *et al.* 1994; Kelce *et al.* 1995; Cooper and Kavlock 1997; Sohoni *et al.* 1998) [19, 34, 45, 69] however, to have an effect, these chemicals must be at concentrations much higher in the vicinity of the receptor than those of the endogenous ligands (Soto *et al.* 1994, 1995) [70, 71]. It is known that some synthetic pesticides, herbicides, polychlorinated biphenyls (PCBs), plasticizers, and surfactant breakdown products have antiestrogenic activity; dioxin has antiestrogenic activity; and polyaromatic hydrocarbons, linuron, vinclozolin, and p, p-DDE have antiandrogenic activity (Cheek and McLachlan 1998) [15].

Altered semen quality has been reported in experimental animals that are exposed to certain dioxins, PCBs, and similar chemicals (Dalsenter *et al.* 1997) [22]. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) decreased sperm count and impacted sperm motility, morphology and acrosome reaction in rats (El-Sabeawy *et al.* 1998) [27]. Chronic exposure of male rats to lindane (including HCH) significantly reduced blood testosterone concentration, epididymal sperm count, and sperm motility (El-Sabeawy *et al.* 1998; Faqi *et al.* 1998) [27, 31], while in guinea pigs, — including HCH — damaged seminiferous tubules and fully arrested spermatogenesis. Reduced testosterone in the blood from conception was also noted in rams exposed to lindane (Beard *et al.* 1999) [7]. Organochlorine pesticides cause reproductive and other endocrine disorders (Beard *et al.* 1999) [7].

In some studies, regarding human exposure to pollutants in the environment, sperm density, motility, and morphology were negatively associated with the concentration of PCB metabolites in seminal plasma (Whorton *et al.* 1979; Cohn *et al.* 1978; Abell *et al.* 2000; Dallinga *et al.* 2002) [80, 3, 18, 21]. However, there was no relation in other studies between changes in sperm parameters and variables of exposure to pesticides (Larsen *et al.* 1998) or PCB in human seminal plasma (Stachel *et al.* 1989; Ensslen *et al.* 1990) [73, 28] Also conflicting were *in vitro* results regarding the effect of PCBs on the motility and acrosome reaction of human spermatozoa (Roediger *et al.* 1989; Hanf *et al.* 1995; Pflieger-Bruss *et al.* 1999; Pflieger-Bruss and Schill 2000) [56, 36, 52,]. There is growing evidence to support the notion that increased pesticide exposure is contributing to adverse reproductive effects (Den Hond *et al.* 2015) [25]. Different classes of pesticides (i.e., organochlorides, organophosphorus compounds, carbamates, pyrethroids, anilides, triazines, etc.) have been shown to be able to affect sperm morphology, count and motility, and to create biochemical and endocrine disorders, increase the incidence of testicular cancer, and compromise fertility in different species (Sengupta and Banerjee 2014) [57].

Effects of pesticides on male reproduction

Testes and Spermatozoa

Environmental pollutants with potential reproductive toxicity present in the seminal plasma of farm animals may have detrimental effects on fertility (Kamarianos *et al.* 2003b, Campagna *et al.* 2009) [43, 13]. Both organochlorine and organophosphorous pesticides can damage germ and somatic cells of the testis, and the effects were genotoxic and teratogenic (Eduardo *et al.* 2001; Bonde *et al.* 2002; Thompson and Bannigan 2008) [26, 76, 9]. Since very little

literature is available on effects of pesticides on semen profile hence human references have been quoted to relate the effects of pesticide residues on seminal profile. Exposure of males to pesticides adversely affects pregnancy outcome through a direct genetic or epigenetic effect on the male germ cells either during spermatogenesis in the testis or sperm maturation in the epididymis, but also by direct exposure to a pesticide in the seminal plasma (Hales and Robaire 2001) [35].

The number of Sertoli cells determines the overall capacity of a male to produce sperm in the adult life, and is fixed during the course of fetal and early neonatal development (Sharpe 1994) [60]. Thus, the disruption of sertoli cell development could have long term consequences for adult reproductive potential (Sharpe and Skakkebaek 1993) [59]. The environmental estrogens influence sperm production in the adult by disrupting the differentiation/multiplication of sertoli cells of the fetal testis during development (Sharpe and Skakkebaek 1993) [59]. Even a transient exposure of pesticides during fetal life resulted in abnormal sertoli cell development, thus affecting sperm production in adult sheep (Sweeney *et al.* 2000) [75]. Adverse effects of pesticide residues exposure on sperm production in rats (Lee *et al.* 1999) [46] and humans (Toppari *et al.* 1996; Carlsen *et al.* 1995) [77, 14] have been reported. However, still there is no evidence of the impact of pesticides on sperm count effects in farm animals (Cameron and Fairnie 1984) [12].

Organochlorine pesticides and PCBs have been found in the semen of human (Dallinga *et al.* 2002) [21] and farm animals (Kamarianos *et al.* 2003a) [43]. The differences observed in the concentration of pollutants found in the semen of farm animals were due to differences in environmental contamination and animal feeds, differences in the permeability of the blood–testis and epididymis barriers, in the metabolism and excretion by the reproductive tract, and the ability of the reproductive system to retain pollutants (Pocar *et al.* 2001) [53].

An inverse correlation was reported between presence of OCPs and OPPs in blood or seminal plasma in one hand and blood testosterone concentrations or semen characteristics on the other (Dallinga *et al.* 2002) [21]. Some of the endocrine disrupting chemicals like chlorpyrifos and piperophos showed an inhibitory effect on testosterone biosynthesis in Leydig cells by altering the expression of some of the crucial steroidogenic enzymes (Viswanath *et al.* 2010) [79].

Organochlorine pesticides detected in bull seminal plasma are detrimental to fertility (Kamarianos *et al.* 2003b) [44]. The presence of environmental pollutants in the fluids surrounding spermatozoa could affect spermatozoa through a variety of mechanisms (Kamarianos *et al.* 2003a) [43]. Spermatozoa density, motility and morphology were negatively influenced by the environmental pollutants (Abell *et al.* 2000) [3]. The spermatozoon, a highly differentiated cell, possesses a condensed, metabolically inactive nucleus and a relatively restricted cytoplasmic compartment. Because the spermatozoa have exceptionally large surface/volume ratio (more than 50:1), they are more prone to negative effects of pesticides. The spermatozoa could well serve as a readily accessible index of the degree of pesticide pollution (Nelson 1990) [48]. The pesticides may affect the outcome of fertilization owing to adverse effects on spermatozoa cell function. However, *in vitro* results concerning the effect of pollutants on the motility, mitochondrial membrane potential and acrosome reaction of spermatozoa have been conflicting (Pflieger-Bruss and Schill 2000) [51]. The pesticide residues can also alter the time required for sperm transport through the epididymis and these are classified as epididymal toxicants (Hess 1998) [39]. The levels of OCPs were higher in the epididymis than in the testis (Cooke *et al.* 2001) [20].

Various reproductive abnormalities expressed as cryptorchidism, hypospadias, and decreased penile length, may be linked to pesticide exposure, most strikingly in maternally exposed boys (Damgaard *et al.* 2006; Andersen *et al.* 2008; Rocheleau *et al.* 2009) [23, 4, 55]. This is significant because male fertility is thought to be declining in many countries (Andersen *et al.* 2008) [4], and perinatal hypospadias/cryptorchidism are risk factors for reduced sperm quality and testicular cancer in adulthood (Skakkebaek *et al.* 2001) [68].

Recent findings in humans have demonstrated the association between exposures to pesticides and the development of obesity (Hatch *et al.* 2010) [38] a factor that may cause increased estrogenic effects on the humans and attenuate Leydig cell function and fertility (Sharpe 2003) [61]. Also in another study, pesticide-exposed workers had increased risk of delivering a boy with cryptorchidism, smaller penises and testicles, lower serum testosterone, and inhibin B, higher serum sex hormone binding globulin (SHBG) and follicle stimulating hormone (FSH), and higher luteinizing hormone: testosterone ratio (Andersen *et al.* 2008) [4].

Sperm count

There is different hypothesis that correlate male infertility with sperm count (Hanke and Jurewicz 2004) [37]; but, in fact, it is not clear whether sperm count changes and other semen parameters influence infertility (Bonde *et al.* 1999) [8]. The ability of the sperm to bind and penetrate oocyte into the pellucid zone is a biological process that predicts a sperm's fertilizing potential (Winker and Rüdiger 2006). The sperm count is related to other seminal features like sperm morphology and motility and these semen quality measures are independent fecundity predictors (Bonde *et al.* 1999) [8]. Some of the epidemiological studies concluded that farmland work increases the risk of specific morphological abnormalities in the sperm of farmers, including decreased sperm counts per ejaculate and decreased percentage of viable sperm (Shojaei Saadi and Abdollahi 2012) [63].

Sperm morphology

It appears pesticides can affect the integrity of DNA; therefore, it is important to evaluate sperm morphology. Contraction of the muscles of the epididymis can lead to immature sperm production. Sperm morphology may be affected by organophosphate pesticides in humans (Yucra *et al.* 2006) [81]. It should be noted that sperm count and morphology measurements are required almost 2 to 3 months after exposure to correlate the exposure to OP with effects on seminiferous tubules and spermatogenesis assessment (Recio-Vega *et al.* 2008) [54].

Sperm concentration

Concentration or density of sperm is referred to as sperm count per millilitre of semen. Although the sperm concentration is the most common factor to be determined in most semen studies (Yucra *et al.* 2006) [81], it is not a male fertility representative (Bonde *et al.* 1999) [8]. OPs (parathion and methyl parathion) have been suggested to be able to affect

sperm concentration by damaging the seminiferous epithelium through proliferation of germ cells (Perry *et al.* 2011) [50]. Sperm concentration below 20×10^6/ml is accompanied by reduced fertility, and the amount below 5×10^6/ml is the clinically significant male infertility threshold in humans (Oliva *et al.* 2001) [49]. Seminal volume does not appear to be directly related to fertility, except in very low volumes. However, sperm concentration may be significantly reduced in high volumes (Oliva *et al.* 2001) [49]. Exposure to pesticides affects sex-accessory glands (Yucra *et al.* 2006) [81]. The seminal vesicles and prostate gland contribute 60% and 30% of the seminal volume respectively and exposure to pesticides affect the production and may also reduce the volume of seminals.

Sperm motility

Sperm motility depends on mid-piece and tail integrity to produce energy for movement. Adenosine triphosphate (ATP) is the principal source of energy for spermatozoa. Protein phosphatases and protein kinases play important role in the process of ATP synthesis. Consequently, any factor that interferes with the assembly of protein components in the tail structure and/or modifies the concentration / function of ATP synthesis may result in reduced sperm motility (Perry *et al.* 2011) [50]. A study by Lifeng *et al.* (2006) [47] showed that the sperm motility could indirectly reflect its ability to fertilize. They found that some reproductive toxicants such as fenvalerate (synthetic pyrethroid) could affect sperm motility at low dose exposures while other reproductive measures such as sperm morphology did not change similarly. Therefore, measurement of sperm motility is a more sensitive method to study male reproductive toxicity (Lifeng *et al.* 2006) [47]. Pesticide exposure is thought to reduce seminal volume and sperm motility, increase seminal pH, and increase abnormal sperm morphology by affecting epididymal, seminal vesicle, and prostate function particularly at head part of spermatozoa (Yucra *et al.* 2006) [81].

References

1. Abdollahi M, Ranjbar A, Shadnia S, Nikfar S, Rezaie A. Pesticides and. oxidative stress: A review. Medical Science Monitor. 2004;10:RA141-47.
2. Abdulhakeem A, Al-majed, Abdulaziz A, Al-Yahya AM, Al-Bakairi AA, Shoeb Q. Reproductive, cytological and biochemical toxicity of yohimbe in male swiss albino mice. Asian Journal of Andrology. 2006;8(4):469-67.
3. Abell A, Ernst E, Bonde JP. Semen quality and sexual hormones in greenhouse workers. Scand Journal Work Environ Health. 2000;26:492-500.
4. Andersen HR, Schmidt IM, Grandjean P, Jensen TK, Budtz-Jørgensen E, Kjærstad MB, *et al.* Impaired reproductive development in sons of women occupationally exposed to pesticides during pregnancy. Environmental health perspectives. 2008 Apr;116(4):566-72.
5. Ansari RA, Rahman S, Kaur M, Anjum S, Raisuddin S. In vivo cytogenetic and oxidative stress-inducing effects of cypermethrin in freshwater fish, Channa punctata Bloch. Ecotoxicology and Environmental Safety. 2011 Jan 1;74(1):150-6.
6. Arabi M, Mohammadpour AA. Adverse effects of cadmium on bull spermatozoa. Veterinary Research Communication. 2006;30:943-51.
7. Beard AP, Bartlewski PM, Chandolia RK, Honaramooz A, Rawlings NC. Reproductive and endocrine function in rams exposed to the organochlorine pesticides lindane and pentachlorophenol from conception. 1999 Mar 1;115(2):303-14.
8. Bonde JP, Hjollund NH, Kolstad HA, Abell A, Larsen SB. Environmental semen studies—is infertility increased by a decline in sperm count. Scandinavian Journal of Work, Environment and Health. 1999;25:12-16.
9. Bonde JP, Joffe M, Apostoli P, Dale A, Kiss P, Spano M, *et al.* Sperm count and chromatin structure in men exposed to inorganic lead: lowest adverse effect levels. Occupational and Environmental medicine. 2002 Apr 1;59(4):234-42.
10. Bonde JP. Environmental Factors. In: Comhaire F H (Ed.) *Male Infertility, Clinical Investigation, Cause Evaluation and Treatment*. Chapman and Hall, London, 1996, 267-84.
11. Bradberry SM, Cage SA, Proudfoot AT, Vale JA. Poisoning due to pyrethroids. Toxicological reviews. 2005 Jun;24:93-106.
12. Cameron AWN, Fairnie IJ. Semen quality, quantity and flock fertility. In: Lindsay D R and Pierce D T (Eds.) *Reproduction in Sheep*. Cambridge University Press, New York, USA; c1984.
13. Campagna C, Guillemette C, Ayotte P, Bailey JL. Effects of an environmentally relevant organochlorine mixture and a metabolized extract of this mixture on porcine sperm parameters *in vitro*. Journal of Andrology. 2009;30(3):317-24.
14. Carlsen E, Giwercman A, Keiding N, Skakkebæk NE. Declining semen quality and increasing incidence of testicular cancer: is there a common cause?. Environmental health perspectives. 1995 Oct;103(suppl 7):137-9.
15. Cheek AO, McLACHLAN JA. Environmental hormones and the male reproductive system. Journal of andrology. 1998 Jan 2;19(1):5-10.
16. Cillo F, de Eguileor M, Gandolfi F, Brevini TA. Aroclor-1254 affects mRNA polyadenylation, translational activation, cell morphology, and DNA integrity of rat primary prostate cells. Endocrine Related Cancer. 2007 Jun 1;14(2):257.
17. Clementi M, Tiboni GM, Causin R, La Rocca C, Maranghi F, Raffagnato F, *et al.* Pesticides and fertility: an epidemiological study in Northeast Italy and review of the literature. Reproductive toxicology. 2008 Sep 1;26(1):13-8.
18. Cohn WJ, Boylan JJ, Blanke RV, Fariss MW, Howell JR, Guzelian PS. Treatment of chlordecone (Kepone) toxicity with cholestyramine: results of a controlled clinical trial. New England Journal of Medicine. 1978 Feb 2;298(5):243-8.
19. Colborn T, Vom Saal FS, Soto AM. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environmental health perspectives. 1993 Oct;101(5):378-84.
20. Cooke GM, Newsome WH, Bondy GS, Arnold DL, Tanner JR, Robertson P, *et al.* The mammalian testis accumulates lower levels of organochlorine chemicals compared with other tissues. Reproductive Toxicology. 2001 May 1;15(3):333-8.
21. Dallinga JW, Moonen EJ, Dumoulin JC, Evers JL, Geraedts JP, Kleinjans JC. Decreased human semen quality and organochlorine compounds in blood. Human

Reproduction. 2002 Aug 1;17(8):1973-9.
22. Dalsenter PR, Faqi AS, Webb J, Merker HJ, Chahoud I. Reproductive toxicity and toxicokinetics of lindane in the male offspring of rats exposed during lactation. Human & experimental toxicology. 1997 Mar;16(3):146-53.
23. Damgaard IN, Skakkebæk NE, Toppari J, Virtanen HE, Shen H, Schramm KW, Petersen JH, Jensen TK, Main KM, Nordic Cryptorchidism Study Group. Persistent pesticides in human breast milk and cryptorchidism. Environmental health perspectives. 2006 Jul;114(7):1133-8.
24. De Celis R, Pedron-Nuevo N, Feria-Velasco A. Toxicology of male reproduction in animals and humans. Archives of andrology. 1996 Jan 1;37(3):201-18.
25. Den Hond E, Tournaye H, De Sutter P, Ombelet W, Baeyens W, Covaci A, *et al.* Human exposure to endocrine disrupting chemicals and fertility: A case–control study in male subfertility patients. Environment international. 2015 Nov 1;84:154-60.
26. Eduardo BO, Niels S, Jose R, John L, Pflieger-Bruss S, Henrik L. Adverse effects of exposure to agropesticides on male reproduction. Acta Pathologica Microbiologica et Immunologica. 2001;109:S233-42.
27. El-Sabeawy F, Wang S, Overstreet J, Miller M, Lasley B, Enan E. Treatment of rats during pubertal development with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin alters both signaling kinase activities and epidermal growth factor receptor binding in the testis and the motility and acrosomal reaction of sperm. Toxicology and Applied Pharmacology. 1998 Jun 1;150(2):427-42.
28. Ensslen SC, Riedel HH, Blüthgen H, Heeschen W. Chlorinated hydrocarbons in seminal plasma and male fertility. Zentralblatt fur Gynakologie. 1990 Jan 1;112(13):817-21.
29. Etta HE, Bassey UP, Eneobong EE, Okon OB. Anti-spermatogenic effects of ethanol extract of Mucuna urens. Journal of Reproduction and contraception. 2009 Sep 1;20(3):161-8.
30. Fao Stat. [cited 2014 Jul 20]; c2014. Available from: http://faostat.fao.org/
31. Faqi AS, Dalsenter PR, Merker HJ, Chahoud I. Reproductive toxicity and tissue concentrations of low doses of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin in male offspring rats exposed throughout pregnancy and lactation. Toxicology and Applied Pharmacology. 1998 Jun 1;150(2):383-92.
32. Favareto AP, Fernandez CD, da Silva DA, Anselmo-Franci JA, Kempinas WD. Persistent impairment of testicular histology and sperm motility in adult rats treated with cisplatin at peri-puberty. Basic & clinical pharmacology & toxicology. 2011 Aug;109(2):85-96.
33. Ford HC, Cooke RR, Kelghtley EA, Feek CM. Serum levels of free and bound testosterone in hyperthyroidism. Clinical endocrinology. 1992 Feb;36(2):187-92.
34. Gray LE, Ostby JS, Kelce WR. Developmental effects of an environmental antiandrogen: the fungicide vinclozolin alters sex differentiation of the male rat. Toxicology and applied pharmacology. 1994 Nov 1;129(1):46-52.
35. Hales BF, ROBAIRE B. Paternal exposure to drugs and environmental chemicals: effects on progeny outcome. Journal of andrology. 2001 Nov 12;22(6):927-36.
36. Hanf V, Behnisch P, Korner W, Sonntag CP, Tinneberg HR, Hagenmaier H. Concentrations and congener profiles of PCB in human preovulatory cervical mucus and effects on sperm motility *in vitro*. Organohalogen Compounds. 1995;26:155-9.
37. Hanke W, Jurewicz J. The risk of adverse reproductive and developmental disorders due to occupational pesticide exposure: an overview of current epidemiological evidence. International journal of occupational medicine and environmental health. 2004 Jan 1;17(2):223-43.
38. Hatch EE, Nelson JW, Stahlhut RW, Webster TF. Association of endocrine disruptors and obesity: perspectives from epidemiological studies. International journal of andrology. 2010 Apr;33(2):324-32.
39. Hess RA. Effects of environmental toxicants on the efferent ducts. Journal of Reproduction and Fertility. 1998;53(Suppl 1):247-59.
40. Hsu PC, Chen MY, Guo YL. Lead-induced changes in spermatozoa function and metabolism. Journal of Toxicology and Environmental Health Part A. 1998 Sep 1;55(1):45-64.
41. Johansson L. Premature acrosome reaction in spermatozoa from lead-exposed mice. Toxicology. 1989 Feb 1;54(2):151-62.
42. Kadavil JA, Katz EJ, Eldefrawi ME, Eldefrawi AT. Modulation of protein phosphorylation and dephosphorylation in neuronal tissue by chlorpyrifos: a novel mechanism of OP toxicity. Toxicologist. 2001;60:243.
43. Kamarianos A, Karamanlis X, Goulas P, Theodosiadou E, Smokovitis A. The presence of environmental pollutants in the follicular fluid of farm animals (cattle, sheep, goats, and pigs). Reproductive Toxicology. 2003a Mar 1;17(2):185-90.
44. Kamarianos A, Karamanlis X, Theodosiadou E, Goulas P, Smokovitis A. The presence of environmental pollutants in the semen of farm animals (bull, ram, goat, and boar). Reproductive Toxicology. 2003b Jul 1;17(4):439-45.
45. Kelce WR, Stone CR, Laws SC, Gray LE, Kemppainen JA, Wilson EM. Persistent DDT metabolite p, p'–DDE is a potent androgen receptor antagonist. Nature. 1995 Jun 15;375(6532):581-5.
46. Lee PC, Arndt P, Nickels KC. Testicular abnormalities in male rats after lactational exposure to nonylphenols. Endocrine. 1999 Aug;11:61-8.
47. Lifeng T, Shoulin W, Junmin J, Xuezhao S, Yannan L, Qianli W, *et al.* Effects of fenvalerate exposure on semen quality among occupational workers. Contraception. 2006 Jan 1;73(1):92-6.
48. Nelson L. Pesticide perturbation of sperm cell function. Bulletin of environmental contamination and toxicology. 1990;45(6):876-82.
49. Oliva A, Spira A, Multigner L. Contribution of environmental factors to the risk of male infertility. Human reproduction. 2001 Aug 1;16(8):1768-76.
50. Perry MJ, Venners SA, Chen X, Liu X, Tang G, Xing H, Barr DB, Xu X. Organophosphorous pesticide exposures and sperm quality. Reproductive Toxicology. 2011 Jan 1;31(1):75-9.
51. Pflieger-Bruss S, Schill WB. Effects of chlorinated hydrocarbons on sperm function *in vitro*. Andrologia. 2000 Sep;32(4-5):311-5.
52. Pflieger-Bruss S, Hagemann S, Heitkamp S, Köhn FM, Körner W, Hanf V, *et al.* Effects of chlorinated hydrocarbons on the acrosome reaction of human

spermatozoa *in vitro*. Hum Reprod. 1999 Jun 1;14:14.
53. Pocar P, Augustin R, Gandolfi F, Fischer B. Octylphenol, an environmental oestrogen, affects oocyte maturation in cattle. InProceedings of the British Society of Animal Science 2001 (Vol. 2001, pp. 64-64). Cambridge University Press.
54. Recio-Vega R, Ocampo-Gómez G, Borja-Aburto VH, Moran-Martínez J, Cebrian-Garcia ME. Organophosphorus pesticide exposure decreases sperm quality: association between sperm parameters and urinary pesticide levels. Journal of applied toxicology. 2008 Jul;28(5):674-80.
55. Rocheleau CM, Romitti PA, Dennis LK. Pesticides and hypospadias: a meta-analysis. Journal of pediatric urology. 2009 Feb 1;5(1):17-24.
56. Roediger B, van der Ven H, Schlebusch H, Wagner U, Knapp M, Al-Hasani S. Einfluss von pestidizen auf die funkion von spermatozoen *in vitro*. Archives of Gynecology and Obstetrics. 1989;245:1041-42.
57. Sengupta P, Banerjee R. Environmental toxins: Alarming impacts of pesticides on male fertility. Human & experimental toxicology. 2014 Oct;33(10):1017-39.
58. Shadnia S, Azizi E, Hosseini R, Khoei S, Fouladdel S, Pajoumand A, Jalali N, Abdollahi M. Evaluation of oxidative stress and genotoxicity in organophosphorus insecticide formulators. Human & experimental toxicology. 2005 Sep;24(9):439-45.
59. Sharpe RM, Skakkebaek NE. Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract?. The Lancet. 1993 May 29;341(8857):1392-6.
60. Sharpe RM. Regulation of spermatogenesis. In: Knobil E and Neill J D (Eds.) *The Physiology of Reproduction*, Raven Press, New York, 2nd Edn, 1994, 1363-34.
61. Sharpe RM. The 'oestrogen hypothesis'–where do we stand now? 1. International journal of andrology. 2003 Feb;26(1):2-15.
62. Shi X, Gu A, Ji G, Li Y, Di J, Jin J, *et al.* Developmental toxicity of cypermethrin in embryo-larval stages of zebrafish. Chemosphere. 2011 Oct 1;85(6):1010-6.
63. Shojaei Saadi H, Abdollahi M. The importance of pesticides effects on human reproduction in farmers. International Journal of Pharmacology. 2 012 Aug 15;8(6):467-469.
64. Sifakis S, Mparmpas M, Soldin OP, Tsatsakis A. Pesticide exposure andhealth related issues in male and female reproductive system. In: Stoytcheva M (Ed.) *Pesticides—Formulations*, Effects, Fate. InTech, Rijeka, Croatia, 2011, 495-526.
65. Singh BP. 'Cancer Deaths in Agricultural Heartland: A study in Malwa Region of Indian Punjab'. M.Sc Thesis, International Institute for Geo-Information science and Earth Observation Enschede, The Netherlands; c2008.
66. Singh PB, Singh V. Bioaccumulation of hexachlorocyclohexane, dichlorodiphenyltrichloroethane, and estradiol-17β in catfish and carp during the pre-monsoon season in India. Fish physiology and biochemistry. 2008 Mar;34:25-36.
67. Sinha N, Narayan R, Shanker R, Saxena DK. Endosulfan-induced biochemical changes in the testis of rats. Veterinary and human toxicology. 1995 Dec 1;37(6):547-9.
68. Skakkebaek NE, De Meyts ER, Main KM. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. Apmis. 2001 Jul;109(S103):S22-30.
69. Sohoni P, Sumpter JP. Several environmental oestrogens are also anti-androgens. Journal of endocrinology. 1998 Sep 1;158(3):327-40.
70. Soto AM, Chung KL, Sonnenschein C. The pesticides endosulfan, toxaphene, and dieldrin have estrogenic effects on human estrogen-sensitive cells. Environmental health perspectives. 1994 Apr;102(4):380-3.
71. Soto AM, Sonnenschein C, Chung KL, Fernandez MF, Olea N, Serrano FO. The E-SCREEN assay as a tool to identify estrogens: an update on estrogenic environmental pollutants. Environmental health perspectives. 1995 Oct;103(suppl 7):113-22.
72. Spira A, Multigner L. The effect of industrial and agricultural pollution on human spermatogenesis. Human reproduction (Oxford, England). 1998 Aug 1;13(8):2041-2.
73. Stachel B, Dougherty RC, Lahl U, Schlösser M, Zeschmar B. Toxic Environmental Chemicals in Human Semen: Analytical Method and Case Studies/Toxische Umweltchemikalien in menschlichem Sperma: Analysenmethode und Fallstudien. Andrologia. 1989 May 6;21(3):282-91.
74. Sundaram K, Witorsch R. Toxic Effects On The Testes. In: Witorsch R (Ed.) *Reproductive Toxicology.* Raven Press, New York, 1995, 99-122.
75. Sweeney T, Nicol L, Roche JF, Brooks AN. Maternal exposure to octylphenol suppresses ovine fetal follicle-stimulating hormone secretion, testis size, and sertoli cell number. Endocrinology. 2000 Jul 1;141(7):2667-73.
76. Thompson J, Bannigan J. Cadmium: toxic effects on the reproductive system and the embryo. Reproductive toxicology. 2008 Apr 1;25(3):304-15.
77. Toppari J, Larsen JC, Christiansen P, Giwercman A, Grandjean P, Guillette Jr LJ, *et al.* Male reproductive health and environmental xenoestrogens. Environmental health perspectives. 1996 Aug;104(suppl 4):741-803.
78. Upasana Ratnakaran. 'Impact of Pesticide residues on fertility in dairy animals'. M.V.Sc. Thesis, Guru Angad Dev Veterinary and Animal Sciences University Ludhiana, India; c2012.
79. Viswanath G, Chatterjee S, Dabral S, Nanguneri SR, Divya G, Roy P. Anti-androgenic endocrine disrupting activities of chlorpyrifos and piperophos. The Journal of steroid biochemistry and molecular biology. 2010 May 1;120(1):22-9.
80. Whorton D, Milby TH, Krauss RM, Stubbs HA. Testicular function in DBCP exposed pesticide workers. Journal of Occupational Medicine. 1979 Mar 1;21(3):161-6.
81. Yucra S, Rubio J, Gasco M, Gonzales C, Steenland K, Gonzales GF. Semen quality and reproductive sex hormone levels in Peruvian pesticide sprayers. International journal of occupational and environmental health. 2006 Oct 1;12(4):355-61.
82. Zeng J, Peng Y, Pan J, Gao H, Wu R, Yin Y, *et al.* Convenient synthesis of micron-sized macroporous polymers with dents on their surfaces and excellent adsorption performance for λ-cyhalothrin. Chemical Engineering Journal. 2015 Apr 15;266:1-1.