



Impact of pesticide residues on fertility of dairy animals: A review

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ABSTRACT

Dairy animals are exposed to a number of pesticides during lifetime. Although, the level of exposure of individual pesticide is usually too low to induce any acute impact on reproductive system, but the combinations of pesticide residues can act additively or synergistically to cause chronic disturbances in hypothalamo-pituitary-gonadal system at all ages and especially in the developing fetus/neonates. The concern is increasing regarding the ability of some pesticide residues, known as endocrine-disrupting chemicals, to disrupt the synthesis, function and metabolism of reproductive hormones. At present, many potential sites of action of pesticide residues have been identified but the knowledge regarding long-term synergistic impact of pesticide residues on reproductive systems is poor especially in dairy animals. The pesticide residues can impair semen quality, as well as female fertility. The present paper identifies the risks to fertility of dairy animals posed by wide spread contamination of our environment with pesticide residues.

Key words: Buffalo, Cattle, Endocrine disruptors, Fertility, Pesticide residues

In recent decades, the number of dairy animals suffering from reduced fertility has risen dramatically as indicated by prolonged calving interval, delayed first ovulation after calving and rise in the number of services per conception (Darwash *et al.* 1999, Petro *et al.* 2010, Ghuman *et al.* 2012). This can be attributed to increasing endocrine imbalance, genetic improvements for milk production, diversion of nutrients for growth and on-farm management (Darwash *et al.* 1999).

These observed trends in fertility parameters might be linked to the effects of environmental pollutants (Petro *et al.* 2010, Rolland *et al.* 2012). Pesticides in the environment may play an important role in contributing to underlying causes of fertility problems in dairy livestock (Kamarianos *et al.* 2003a, 2003b, Campagna *et al.* 2009). In fact, throughout the previous century, the production, use and as a result the presence of pesticides in the environment has increased enormously. Consequently, animals are exposed to a much larger amount and a variety of pesticides. Although an individual component of a cocktail of pesticide residues may have no observable biological impact, but additive or synergistic impact of each residue in the mixture can exert

significant damage. It is emerging that low-level, 'real-life' mixtures of pesticide residues may carry significant biological potency (Fowler *et al.* 2012). This makes critically important to have awareness regarding the biological responses of pesticide residues (Rajapakse *et al.* 2002). In a recent field study, about 15.1 to 17.5% dairy animals of low-pesticide usage and high-pesticide usage area were detected positive for pesticide residue(s), and the blood serum concentrations of pesticide residues were high in dairy animals of the high-pesticide usage area compared to low-pesticide usage area (27.5±21.0 vs 65.6±68.5 ng/ml; Ratnakaran *et al.* 2012a).

Since the biological system is affected by the pesticide residues and the process of development is very similar among all the vertebrates, therefore, these residues may cause similar adverse effects in animals (Fig.1.). Pesticide residues may cause disruption of thyroid function (Hansen *et al.* 1998, Langer *et al.* 1998), immune system (Vine *et al.* 2000) and sexual differentiation of brain during fetal development (Moore *et al.* 2001). All these effects have the potential to compromise the (re)productivity of domestic animals through improper functioning of the reproductive axis (Birnbaum 1995). Moreover, within the food chain, the concentration of pesticide residues increases from one trophic level to the next (Nimrod and Benson 1996). Humans are on the highest trophic levels and have highest concentration of these residues. Thus, pesticide residues can induce changes in organ structure or function in the subsequent unexposed generations

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(Bogh *et al.* 2001, Anway and Skinner 2006, Edwards and Myers 2007, Steinberg *et al.* 2008).

The published data in female of a species indicated that reproductive functions can be compromised by exposure to pesticides resulting in infertility (Mattison 1985, Thomas 1993). In females the pesticide exposure induced alterations which include poor reproductive behavior, sub-fecundity, infertility, pregnancy loss, growth retardation, intra-uterine fetal demise and ovarian failure (Sharara *et al.* 1998, Bhatt 2000, Zama and Uzumcu 2010). In fact, dairy cattle rearing on drinking water contaminated with sewage reduced their reproductive performance (Meijer *et al.* 1999).

The exposure of males to pesticides can adversely affect pregnancy outcome through a direct genetic or epigenetic effect of their residues on the male germ cells either during spermatogenesis in the testis or sperm maturation in the epididymis, or by the direct exposure of oocyte during fertilization to the pesticide residues in the seminal plasma (Eduardo *et al.* 2001, Bonde *et al.* 2002, Thompson and Bannigan 2008). This paper will review in detail the impact of pesticide residues on reproductive functioning of dairy animals.

Interference with hormone function

There is growing evidence regarding the adverse impact of certain pesticide residues on reproductive system, and such pesticide residues are known as 'reproductive toxicants' or 'endocrine disrupters'. These toxicants modulate and/or disrupt reproductive hormone milieu by acting at a variety of sites including hypothalamus, pituitary and reproductive organs (Colborn *et al.* 1993, Sweeney 2002).

Like hormones, small amounts (parts per trillion) of 'endocrine-disrupting chemicals (EDCs)' may affect the endocrine system of living-beings (Gandolfi *et al.* 2002, Petro *et al.* 2010). The half lives of endocrine-disrupting pesticides in the environment range from days to years. Many EDCs [(poly-aromatic hydrocarbons (PAH), poly-chlorinated biphenyls (PCBs), poly-brominated diphenyl ethers (PBDEs)] are highly persistent and have half lives >10 years (Smith 1995). Alkyl phenols have a half life of <10 days, therefore, the risk of ingestion and accumulation by farm animals is relatively low. The half lives of phthalates generally range from weeks to months, which allow significant animal exposure since concentrations in sheep liver is substantially elevated relative to environmental levels (Boerjan *et al.* 2002).

Endocrine-disrupting organochlorine or organophosphate pesticides (OCPs or OPPs) may interfere with the synthesis, secretion, transport, metabolism, binding, action or elimination of endogenous hormones that are responsible for homeostasis, reproduction and developmental processes (Colborn *et al.* 1993, Kavlock and Ankley 1996). The pesticide residues may impact by: (a) altering enzyme pathways involved in hormone synthesis, (b) mimicking

hormone functions, (c) blocking hormone receptors, (d) altering hormone receptor number, (e) changing hormone-receptor affinity, and (f) varying hormone metabolism (Gore *et al.* 2006, Gore 2008).

Various EDCs can act like endogenous steroids, viz. estrogenic, anti-androgenic, androgenic or anti-estrogenic (Harris *et al.* 1997, Kelce *et al.* 1998), through their interaction with nuclear receptors, membrane receptors, neurotransmitter receptors and orphan receptors (Foster *et al.* 2004). The degree of affinity of EDCs for hormone receptors is variable but is always much lower than the affinity of natural steroids by a factor of 100–1,000. Some disrupting effects of EDCs in the hypothalamus, pituitary and ovary, and on oocyte and sperm are expressed through mechanisms that do not involve binding to these receptors (Thomas *et al.* 1999). The accumulation of EDCs in glandular tissue can lead to cell death and necrosis, which is ultimately responsible for reduced hormone production (Schettler *et al.* 2003).

Endocrine-disrupting chemicals are biologically significant as they can exert effects on physiological systems at very low concentrations with orders of magnitude lower than those known to have acute toxic effects (Brevini *et al.* 2005, Fowler *et al.* 2007). Furthermore, these individual pesticide residues acting as EDCs are present in low concentration in the environment, but due to their properties such as long environmental half-life, low water solubility and high lipid solubility, they are bio-accumulated and bio-magnified in the animal/human adipose tissue and hence become dangerous in the view of their synergistic and/or additive effect (Rhind 2002, Petro *et al.* 2010). Actually, in dairy animals of a slaughterhouse, as well as low-pesticide usage and high-pesticide usage areas of Punjab state, the proportion of blood samples positive for >1 pesticide residue was 66.7%, 25.0% and 16.7%, respectively (Ratnakaran *et al.* 2012b, c).

Impact during fetal and neonatal period

In addition to the direct impact of endocrine disruptors on fertility outcome, the other major issue is trans-generational epigenetic impact at low doses during *in utero* gonadal development (Manikkam *et al.* 2012). The fetal exposure to EDCs can impair reproductive outcomes that are evident from growing biological, toxicological, experimental and human exposure data (Rolland *et al.* 2012).

Many reasons are reported for the higher impact of pesticide residues on the developing fetus and the post-natal offspring. The endocrine system has major control on the growth and development of fetus, thus fetal exposure to pesticides during this phase may lead to lifelong health and reproductive abnormalities, either overt or subtle (Bern 1992). This situation is aggravated by the fact that the fetus seems to be a preferential site for environmental pollutants. During fetal life, the significant partitioning between the

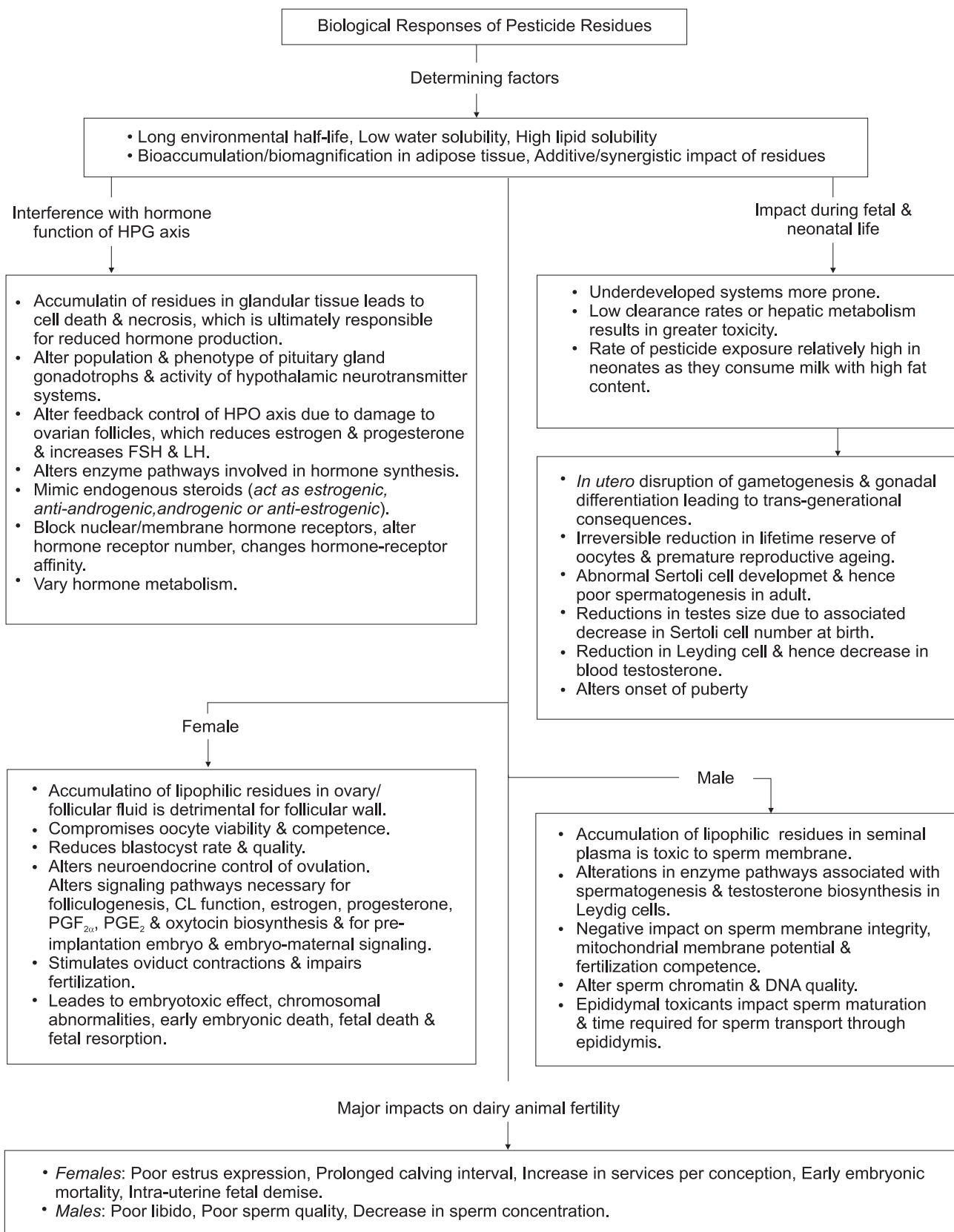


Fig. 1. Diagrammatic representation of impact of pesticide residues on fertility of dairy animals.

maternal and fetal compartments leads to pesticide deposition in the lipid-rich tissues of the fetus, where poor enzymatic activity increases the accumulation of lipophilic pesticide (Waliszewski *et al.* 2000). Also, the metabolism of pesticide residues in the fetus is particularly low due to their poor binding affinity for the sex hormone binding globulin (McLachlan and Arnold 1996). Moreover, the neonates have a higher percentage of total body water and less body fat to serve as storage sites for lipophilic pesticides (Olhager *et al.* 2003). Thus, less deposition of pesticide residues leads to their higher circulating levels in the newborn. Additionally, low clearance rates or hepatic metabolism may result in greater toxicity in the fetus and neonate. In fact, offspring at birth had dioxins concentrations that were 25% higher than those circulating in the mother (Pocar *et al.* 2003).

Much of the current evidence suggested that the mammalian reproductive system is susceptible to an alarming impact of detrimental EDCs during the fetal and neonatal life than in the adulthood (Majdic and Saunders 1998). Following a low dose exposure to pesticides throughout the life of mother, there is accumulation of lipophilic pesticides in the fat stores (Ekelund *et al.* 1990, Ahel *et al.* 1993). These fat stores are rapidly mobilized during the energetically expensive periods of pregnancy and in particular lactation, and exert endocrine disrupting effects in late pregnancy and post-natal life (Bigsby *et al.* 1997). During 2–3 weeks post-partum, an increase in PCB concentrations observed in milk samples from high producing dairy cows indicated the role of massive lipolysis in liberating and thereby increasing EDC concentrations in milk (Petro *et al.* 2010). The slow metabolism or excretion of unusually high concentrations of pesticides during fetal and post-natal life leads to underdevelopment of reproductive and immune axes. Moreover, these underdeveloped systems in the developing fetus are more prone to adverse impact of pesticides (Crisp *et al.* 1998). The risk of pesticide exposure through feed for young animals is different from the adult because they consume milk and owing to the high fat content of milk the rates of pesticide exposure are relatively high (Dekoning *et al.* 2000, Petro *et al.* 2010). Studies have shown that neonatal calves are able to absorb EDCs added to the colostrum even by 1 h post-partum (Keller *et al.* 2001).

The developmental stage at which damage occurs determines the impact that the exposure to pesticide residues will have on reproduction (Hoyer 2005, Uzumcu and Zachow 2007). In fact, *in utero* disruption of gametogenesis and gonadal differentiation can have trans-generational consequences (Anway and Skinner 2006).

Impact on hypothalamic-pituitary axis

Majority studies have found the sensitive windows viz. development, gestation and lactation for the irreversible impact of pesticide residues on hypothalamic-pituitary axis (IPCS 2002). On reproductive system, the endocrine

disrupting effects of pesticide residues could be through their binding to estrogen, androgen or other receptors. Thus, at hypothalamus, pituitary and at reproductive organs, various endocrine disruptors may act either like endogenous steroids or can block the action of endogenous steroids (Zama and Uzumcu 2010). These pesticide residues can cause imbalance between estrogen and androgens, which ultimately influences the hypothalamic–pituitary–gonadal axis (Rivas *et al.* 2002).

In females, an extensive damage to ovarian follicles disrupts the endocrine balance leading to a reduction in circulating estrogen and progesterone and an increase in follicle stimulating hormone (FSH) and luteinizing hormone (LH). In an *in vitro* study, progesterone and estrogen release was decreased after the exposure of bovine granulosa cells to OCPs at 0.0001 to 1.0 parts per billion (ppb) (Faundez *et al.* 1996). Various endocrine-disrupting pesticide residues can indirectly change the balance of feedback control of hypothalamus-pituitary-ovarian system (Pocar *et al.* 2003). Also, the secretion of oxytocin from granulosa and luteal cells can be increased by the PCBs (Mlynarczuk *et al.* 2005, Mlynarczuk and Kotwica 2006).

In males, an inverse correlation exists between the presence of OCP and OPP residues in blood or seminal plasma on one hand and blood testosterone concentrations or semen characteristics on the other (Dallinga *et al.* 2002). Pesticides like chlorpyrifos and piperophos have inhibitory effect on testosterone biosynthesis in leydig cells by altering the expression of crucial steroidogenic enzymes (Viswanath *et al.* 2010). In a study, the exposure of pesticides during fetal life inhibited mid-gestational rise in gonadotropin secretion, and hence about 40% reductions in testes size was observed due to associated decrease in Sertoli cell number at birth in sheep (Sharpe *et al.* 1995).

Pesticide exposure associated estrogenic effects may attenuate Leydig cell function, fertility and leads to obesity in males (Sharpe 2003, Hatch *et al.* 2010). Pesticide-exposed greenhouse workers exhibited high risk of delivering a male with cryptorchidism, smaller penis and testicles, lower serum testosterone, and inhibin-B, higher serum FSH, and higher LH:testosterone ratio (Damgaard *et al.* 2006, Andersen *et al.* 2008, Rocheleau *et al.* 2009).

The fetal exposure to a cocktail of environmental pollutants via maternal ingestion is able to disrupt fetal hypothalamic and pituitary *KISS-1* mRNA expression, as well as the proportion of kiss-peptin positive cells in the fetal pituitary that are also positive for LH receptor and estrogen receptor (Navarro and Tena Sempere 2008, Smith *et al.* 2005, Bellingham *et al.* 2009). The kiss-peptin/GPR54 system is a potential target for the pesticide residues to alter the puberty timing, which reflects the activation of hypothalamus-pituitary axis, and reproductive function (Bellingham *et al.* 2009). Similarly, in female lambs, only *in utero* exposure, but not the pre-pubertal exposure, to octylphenol is able to alter onset of puberty and pattern of FSH release during the

late follicular phase (Wright *et al.* 2002, Evans *et al.* 2004).

To address the real world exposure of pollutants, the adult ewes were maintained on sewage sludge irrigated pastures, that lead to alterations in: (a) population and phenotype of pituitary gland gonadotrophs, and (b) activity of various neurotransmitter systems within the hypothalamus (Bellingham *et al.* 2009). Thus, EDC induced alterations in the hypothalamus-pituitary axis have deleterious impact on gonadal function and, ultimately fertility.

Impact on testes and spermatozoa

Pesticide residues can be detrimental to male reproductive system by causing toxicity to sperm plasma membrane as many lipophilic OCP and PCB residues have the ability to concentrate in seminal plasma and were detected in the seminal plasma of farm animals (Kamarianos *et al.* 2003b, Campagna *et al.* 2009). Their concentrations were variable among the farm animals due to differences in animal feed, permeability of the blood-testis and blood-epididymis barrier, and the ability of the reproductive system to metabolize and excrete pollutants (Pocar *et al.* 2001).

The presence of pesticide residues in the fluids surrounding spermatozoa have negative influence on spermatozoa cell function, viz. spermatozoa density, motility and morphology (Abell *et al.* 2000, Kamarianos *et al.* 2003a). Organophosphorus pesticide residues can alter spermatozoa chromatin structure and DNA quality at different stages of spermatogenesis, and by disrupting the hypothalamic-pituitary-gonadal axis (Recio *et al.* 2005, Salazar-Arredondo *et al.* 2008). In fact, spermatozoa are more prone to negative effects of pesticide residues due to their exceptional large surface D volume ratio (>50:1; Nelson 1990). However, *in vitro* results are conflicting regarding the impact of pollutants on motility, mitochondrial membrane potential and acrosome reaction of spermatozoa (Pflieger-Bruss and Schill 2000).

Endosulfan exposure has the capacity to impair testicular functions by causing alterations in enzyme pathways associated with spermatogenesis (Sinha *et al.* 1995). The exposure of buffalo spermatozoa to pesticides (chlorpyrifos and endosulfan) negatively affected the spermatozoa plasma membrane integrity, mitochondrial membrane potential and fertilization competence (Selvaraju *et al.* 2011). Although these pesticides caused spermatozoa dysfunctions at a higher concentration than that present in the environment, their influence on male infertility in farm animals could not be ruled out owing to their cumulative effect or their chronic exposure (Selvaraju *et al.* 2011).

Epididymal toxicant, a class of pesticide residues, has impact on the time required for sperm transport through the epididymis (Hess *et al.* 1998). Moreover, higher concentrations of OCP residues were observed in the epididymis than in the testes (Cooke *et al.* 2001).

During the course of fetal or early neonatal life, any disruption in the differentiation/multiplication of Sertoli cells

in fetal testis by the environmental estrogens is detrimental for the adult reproductive potential because the capacity of an adult to produce sperm is determined by the Sertoli cells (Sharpe and Skakkebaek 1993, Sharpe 1994, Pocar *et al.* 2001, Boerjan *et al.* 2002). Even a transient exposure of pesticides during fetal life was associated with abnormal Sertoli cell development and poor sperm production in the adult sheep (Sweeney 2002). *In utero* and lactational exposure of male rats to an environmentally relevant mixture of OCPs can exert anti-androgenic impact during testicular development (Anas *et al.* 2005), thus suggesting a potential reproductive hazard for humans and other species. The ewes reared on pastures fertilised with sewage sludge gave birth to offsprings that exhibited a decrease in foetal blood testosterone levels as well as reduction in Leydig, Sertoli and germ cell numbers (Paul *et al.* 2005). Main pathogenic mechanism of toxic effect of pesticide residues on testes is damage of Sertoli/Leydig cells, that leads to disturbed spermatogenesis and testosterone inhibition (Ozmen and Mor 2012).

Adverse impact of pesticide exposure was reported on sperm production in rats (Lee *et al.* 1999). Limited studies conducted on farm animals have not confirmed the impact of pesticide exposure on sperm count (Cameron *et al.* 1984). In fact, male fertility is declining in many countries and perinatal hypospadias and cryptorchidism are associated risk factors for reduced sperm quality and testicular cancer in adulthood (Shakkebaek *et al.* 2001, Andersen *et al.* 2008). Impairment in testicular descent, spermatogenesis and sexual function was observed in the offspring born from anti-androgenic pesticide exposed dams (Pocar *et al.* 2001, Boerjan *et al.* 2002).

Joffe (2010) suggested that in addition to fertility outcomes, the semen quality can be used as an early biomarker of gamete deterioration. In fact, semen quality was found surprisingly correlated to life expectancy in the next generation's health (Jensen *et al.* 2009).

Impact on ovary and oocyte

Chronic exposure to environmental pollutants leads to their accumulation in body fat, blood serum and consequently in follicular fluid (Wolff 1983). In fact, the follicle basement membrane is permeable to low and high molecular weight substances that can diffuse into follicular fluid (Edwards 1974). However, the kind of contamination and the concentration of contaminants in follicular fluid may vary according to the geographic area.

In a recent study, the presence of pesticide residues in high concentrations in buffaloes subjected to slaughtering was suggested as the causative factors underlying their infertility scenario. About 36.2% blood samples, 58.6% ovarian tissue samples and 21.4% ovarian follicular fluid samples of the buffaloes subjected to slaughtering were detected positive for pesticide residues (Ratnakaran *et al.*

2012b, 2012c). Moreover, blood, ovarian and follicular fluid concentrations of pesticide residues were alarmingly high (211.0 ± 284.0 , 245.10 ± 330.3 and 526.1 ± 617.4 ng/ml, respectively) in slaughtered buffaloes compared to dairy animals of low-pesticide usage and low-pesticide usage areas (27.5 ± 21.0 to 65.6 ± 68.5 ng/ml) of Punjab state (Ratnakaran *et al.* 2012b, 2012c). Intriguingly, residues of some pesticides viz. DDT, endrin, endosulphan and butachlor were high in ovarian tissue in comparison to their blood concentrations. Nevertheless, EDC concentrations in serum were regraded as reliable predictors of the contamination status of the follicular micro-environment (Petro *et al.* 2012).

The ovarian follicle is a very fragile micro-environment where interactions between hormones, growth factors, the oocyte and its surrounding somatic cells are essential to generate a fully competent oocyte (Petro *et al.* 2012). However, the ability of lipophilic OCPs to concentrate in the bovine ovary and in follicular fluid makes them detrimental for granulosa cells (Faundez *et al.* 1996). As the follicular cells surround oocytes at all stages of development, the pesticide residues may cause alterations within the follicular wall, which compromise its ability to maintain oocyte viability (Buccione *et al.* 1990). Oocyte destruction can result from toxic pesticide residues by directly impairing the viability of oocyte and, thus resulting in impaired fertility (Kamarianos *et al.* 2003a). Lipophilic pesticides due to their physical and chemical properties associate with lipid and protein components in plasma membrane, and hence affect oocyte structure and function (Rogers *et al.* 1976). An overall higher EDC contamination in the follicular micro-environment was associated with a decreased fertilization rate and consequently with a lower chance of an oocyte to develop into a high-quality embryo (Petro *et al.* 2012).

The exposure of ovary to pesticides can have temporary or permanent impact on fertility depending upon the developmental stage at which damage occurs (Uzumcu and Zachow 2007). The pesticides having selective impact on growing or antral follicles have temporary influence on reproductive functions as these follicles are replaced by recruitment of other follicles (Davis *et al.* 1994). However, the pesticides that extensively destroy oocytes contained in the primordial and primary follicles have a permanent effect on reproduction (Hooser *et al.* 1994).

The effects on oocyte caused by a repeated low-dose exposure of female mice to PAH residues were 250 times more toxic than a single high-dose (Borman *et al.* 2000). A mixture of pesticides @ 1.0 ng/ml exerted adverse impact on bovine oocyte maturation and embryonic development (Pocar *et al.* 2001). The maturation stages of oocyte and early embryo have susceptibility even towards background doses of pesticide residues (Pocar *et al.* 2001). The exposure of pig oocytes to an OCP mixture during *in vitro* maturation negatively affected the maturation rate without increasing degeneration rate. Moreover, quality and viability of cumulus

cells decreased, developmental competence of exposed oocytes got affected and the blastocyst rate and quality reduced in a dose-dependent manner (Campagna *et al.* 2001, 2002).

The pesticide exposure during neonatal period causes damage to primordial follicles (germ cells) in ovaries thus producing irreversible form of infertility (sterility) in the adult dairy animals (Pocar *et al.* 2001, Boerjan *et al.* 2002). This pesticide exposure during early development can irreversibly reduce the female's lifetime reserve of oocytes, as the rapidly dividing (meiosis) primordial germ cells and oogonia during this period are highly sensitive to destruction by pesticide residues (Hoyer and Sipes 1996, Pocar *et al.* 2005). In female rodent offspring, *in utero* exposure of tetrachlorodibenzo-p-dioxin (TCDD) lead to permanent reduction of ovarian mass and corpora lutea, premature aging of ovaries and hence, early decline in fertility and fecundity (Gray *et al.* 1997, Wolf *et al.* 1999). The prenatal exposure of female mice to dioxin-like PCBs reduces the number of germ cells in the ovaries by 40–50%. This decrease appeared in all stages of oocytes and follicles and lead to premature reproductive ageing (Ronnback and de Rooij 1994).

Impact on estrus cycle

In adult cyclic females pesticide exposure manifests infertility. This was suggested by observations of a study in which reproductive disorders (anestrous and repeat breeder) in dairy animals were associated with serum pesticide residue concentrations (Ratnakaran *et al.* 2012d). In a field study in Punjab state, higher proportion of dairy animals reared in low-pesticide usage area were showing regular estrus cycles followed by successful conception, whereas, the proportion of animals exhibiting irregular estrous, anestrous or repeat breeding were more in high-pesticide usage area (Ratnakaran *et al.* 2012d). Moreover, the proportion of dairy animals positive for pesticide residues as well as suffering from repeat breeding syndrome was high (24%) along with high serum pesticide residues (70.1 ± 82.8 ng/ml). In contrast, the proportion of animals positive for a pesticide residue and exhibiting pregnancy was less (4%), and their serum pesticide residues were low (11.8 ± 0.5 ng/ml; Ratnakaran *et al.* 2012e). Furthermore, the major proportion of pesticide residue positive estrus cyclic, anestrous and repeat breeder animals were present in the high-pesticide usage area and had higher serum pesticide residues compared to their counterparts in low-pesticide usage area (Ratnakaran *et al.* 2012f). These findings in pregnant animals in comparison to repeat breeder animals suggested that serum pesticide residues might play a role in the occurrence of reproductive disorders.

Furthermore, the disruptive impact of pesticide residues on estrus cycle activity of dairy cattle was shown when a transient exposure of an OCP, malathion at the onset of estrus led to inhibition of progesterone secretion and poor conception rate (Prakash *et al.* 1992). Moreover, a transient

exposure to pesticides on the day of estrus altered neuroendocrine control of ovulation (Stoker *et al.* 2001). The effects of pesticide residues on estrogen receptors and interference in prostaglandin biosynthesis are the factors responsible for prolongation of estrous cycle, delay in LH surge and thus, an increase in early embryonic mortality (Stoker *et al.* 2001, Boerjan *et al.* 2002).

Many effects of pesticide residues leading to an impact on estrous cycle are mediated through signaling pathways involving aryl-hydrocarbon receptor (AhR) which is a binding partner of dioxins and coplanar PCBs (Huang 2008, Nakanishi 2008). These pathways are essential for fertility due to their role in folliculogenesis, estrogen and progesterone biosynthesis and signaling and corpus luteum function (McMillan and Bradfield 2007, Ohtake *et al.* 2009). From ovary, the release of progesterone and estrogen decreased after exposure of bovine granulosa cells to OCPs at 0.0001 to 1.0 ng/ml (Faundez *et al.* 1996).

The course of estrous cycle in cattle is affected by DDT and its metabolites through impaired release of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), PGE_2 and oxytocin from the uterus (Wrobel *et al.* 2009). During luteolysis, the positive feedback loop existing between $PGF_{2\alpha}$ and oxytocin was altered by DDT and its metabolites (Flint *et al.* 1990, Silvia *et al.* 1991). Pesticides, viz. DDT and PCBs have affinity to estradiol receptors and they can stimulate estradiol-dependent $PGF_{2\alpha}$ and inhibit PGE_2 secretion from the endometrium. Moreover, they stimulate estradiol-dependent oxytocin secretion from luteal and granulosa cells (Wrobel *et al.* 2009). In addition, the exposure to PCBs as well as to DDT and its metabolite (DDE) can alter the synthesis of oxytocin precursor (oxytocin-neurophysin-I), and the terminal enzymes involved in post-translational processing of oxytocin synthesis (Mlynarczuk *et al.* 2009).

During the course of estrous cycle, PCB induced alteration in the ratio of $PGF_{2\alpha}$: PGE_2 is crucial for the establishment of early pregnancy (Niswender *et al.* 2000, Parent *et al.* 2003). The PCBs and their hydroxylated derivatives can stimulate the secretion of both $PGF_{2\alpha}$ and PGE_2 from the oviductal epithelial cells within short period of time (2h), which ultimately stimulates the contractions of oviduct and, hence impairs fertilization (Wrobel *et al.* 2010). Also, PCBs could affect the contractions of non-pregnant uterus (Wrobel *et al.* 2005), but they did not influence steroidogenesis in granulosa and luteal cells (Mlynarczuk *et al.* 2005, Mlynarczuk and Kotwica 2006).

Impact on embryo

Embryo implantation is highly vulnerable to pesticide residue induced endocrine disruption even for an exposure period as short as 4 h at concentrations as low as 10nM or 2 ng/kg (Rhind *et al.* 2010). Pesticide residues can interfere with the actions of many hormones and receptors essential for embryo implantation and embryo development (Rhind

et al. 2010). It was suggested that any event that delays ovulation results in chromosomal abnormalities and early embryonic death. Pesticide residues that delay the LH surge were associated with increased fetal loss in rats (Stoker *et al.* 2001). There is also the potential for pesticide residues to speed up the rate of embryo transport through the oviduct, thus preventing implantation because of insufficient time for uterine preparation (Cummins *et al.* 1990). In cattle, an environmentally relevant mixture of over 60 PCB congeners affected oocyte maturation, fertilization and embryo development at doses ranging between 0.001 and 1 mg/ml, the minimum effective dose (0.001 mg/ml) being approximately 10-fold lower than the mean level found in human follicular fluid in non-exposed women (Trapp *et al.* 1984, Pocar *et al.* 2001).

Pre-implantation exposure to pesticides can perturb energy metabolism (Tonack *et al.* 2007) and down regulate the relevant genes (Hanlon *et al.* 2005). Also, the direct embryotoxic effects of pesticide are possible through actions on hormone receptors (Agras *et al.* 2007, Davey *et al.* 2007). Other effects of pesticides on pre-implantation embryos and after implantation are intermediated through various signaling pathways (Huang 2008, Nakanishi 2008). These pathways are necessary for the normal development of pre-implantation embryo and embryo-maternal signaling during implantation (Clausen *et al.* 2005).

Organophosphorus pesticides have the potential to cause fetal death and increase the early resorption (Mlynarczuk *et al.* 2010). The frequency of abortions was correlated with the presence of DDT or PCBs in the tissues of cows (Macklin and Ribelin 1971) even though blood plasma concentrations of ovarian steroids were not changed (Leoni *et al.* 1989). The early exposure to pesticide residues may contribute to a spectrum of diseases throughout life involving intra-uterine growth retardation, disorders of ovulation, metabolic syndrome and sensitivity to cancer (Parent *et al.* 2003, Weselak *et al.* 2007).

Future strategies

Collectively, the potential reproductive hazard to world's dairy industry due to growing environmental contamination with pesticides is generating concern among the scientists and policy makers. In Punjab (India), studies have found high concentrations of pesticide residues (0.03–0.98 ppm) in milk and meat of farm animals including cattle, buffalo and goat (Aulakh *et al.* 2006, Battu *et al.* 2004, Bedi *et al.* 2005). But, even in the presence of huge contamination of our environment with pesticides, till date well-planned studies have not been carried out to rule out whether or not widespread overuse of pesticides in India is one of the etiological factors responsible for the occurrence of various reproductive disorders in dairy animals. However, research in developed countries has shown that environmental contamination of pesticides does produce these effects. This

is intriguing in the sense that contamination of our environment with the pesticides or reproductive toxicants is extremely high compared to the developed countries (Mathur *et al.* 2005). Thus, it would be essential to fully appreciate and assess the detrimental impact of pesticides on the reproductive function of dairy animals for assessing the role of pesticides in causing various fertility problems. To save the future of our dairy industry, it is essential to identify the pesticides acting as reproductive toxicants now, otherwise it will be too late. Therefore, we need to solve the critical issue — whether and to what extent the reproductive performance of dairy animals is affected by pesticide accumulation in their body. Our results regarding the impact of pesticide residues on fertility of dairy animals emphasized the need to sustain research efforts on this issue as these aspects strengthen the need to implement gamete quality monitoring systems. Moreover, these adverse impacts of pesticides warrant that farmers should be educated about judicious use of pesticides and monitoring of pesticide usage needs to be strengthened through control sale of pesticides.

Particular attention must be paid to increasing contamination of environment with pesticides, in order to minimize the risks to livestock and consumers. Continued monitoring and periodic reassessment of risks posed by these pollutants is desired to develop strategies to prevent infertility issues of dairy animals arising from the use of various pesticides. Nevertheless, we need to be concerned regarding the changes in physiology of reproductive function that may be expressed in subsequent generations after exposure to pesticide residues.

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