REVIEW

The impact of endocrine disruptor chemicals on oocyte/embryo and clinical outcomes in IVF

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Abstract

The negative impact of endocrine-disrupting pesticides on human fertility is now a key issue in reproductive health. There are much fewer literature data about the impact of pesticide exposure on women than on men and very few studies of women participating in an *in vitro* fertilization (IVF) programme. In the present review, we found that (1) various pesticides with an endocrine-disrupting action are associated with poor oocyte maturation and competency, embryonic defects and poor IVF outcomes, and (2) some pesticide compounds are linked to specific causes of female infertility, such as premature ovarian insufficiency, polycystic ovarian syndrome, and endometriosis. IVF participants living in agricultural regions should be informed about the fertility decline, low ongoing pregnancy rates, and elevated risk of miscarriage associated with exposure to high doses of pesticides.

Key Words
- endocrine disruptor
- oocyte-embryo
- clinical outcome
- IVF

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Introduction

Infertility is defined as failure to obtain a clinical pregnancy after 12 months of regular, unprotected sexual intercourse. On average, it affects 8–12% of couples of child-bearing age (1). A decline in human fertility has prompted an increasing proportion of couples to enrol in *in vitro* fertilization (IVF) programmes. Over the last 50 years, the sperm count has fallen by 32–50% in Europe and United States (2, 3); this fall is too rapid to be due to a genetic factor but might be related to one or more environmental factors, such as exposure to pesticides.

Pesticide are substances or combinations of substances used in many areas of agriculture and industry. They include (1) phytosanitary products (also referred to as phytopharmaceuticals) used in agricultural or non-agricultural plant sectors to control insects (insecticides), weeds (herbicides), fungi and moulds (fungicides), or pests (rodenticides); (2) biocides used in industry (treatment of wood and textiles), hospital environments (hydroalcoholic gels), and domestic settings (disinfection); and (3) antiparasitics used in human and veterinary medicine (treatment of lice, scabies, mites, fleas, ticks, etc.) (4, 5).

The main families of pesticides by chemical composition are as follows:

- **Organophosphorus** (OP) compounds, such as chlorpyrifos, cypermethrin, malathion, and parathion
- **Organochlorine** (OC) compounds, such as methoxychlor (MXC), 2,3,7,8-tetrachlorodibenzo-pdioxin (TCDD), dichlorodiphenyldichloroethane (DDD), dichlorodiphenyldichloroethylene (DDE), dichlorodiphenyltrichloroethane (DDT), polychlorinated biphenyls (PCBs), dieldrin, endosulfan, hexachlorobenzene (HCB), hexachlorocyclohexane (HCH), and lindane
- **Carbamates**, such as carbofuran, benomyl, and mancozeb
- **Pyrethroids**, such as permethrin
- **Triazines**, such as atrazine

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Many pesticides are known to be endocrine-disrupting chemicals (EDCs), defined as 'exogenous agents, that are potentially capable of synthesis, secretion, transport, binding, action, or elimination of the natural hormones responsible for the maintenance of homeostasis, reproduction, and developmental processes in the body' (5). Even though the involvement of EDCs in certain reproductive diseases is well documented, only few studies have examined the direct impact of pesticides on fertility (and especially in female infertility) in IVF programmes. Hence, the objective of the present review was to assess the impact of the most frequently used pesticides on female fertility, oocyte-embryo quality, and clinical outcomes in IVF programmes.

Pesticides as EDCs

A large body of evidence from animal studies and epidemiological surveys shows that pesticides like bisphenol A (BPA) and phthalates have an endocrine-disrupting action and a reprotoxic impact (5, 6, 7, 8, 9, 10, 11). Furthermore, Manikkam (12) highlighted the existence of a transgenerational risk in the rat: *in utero* exposure of pups to a mixture of pesticides (administered by gavaging the pregnant dams) was associated with changes in puberty onset, spermatogenesis, and the development of ovarian follicles up to the F3 generation.

The direct, toxic endocrine-disrupting effect (i.e. a dose-response relationship) has rarely been characterized for a single target compound. In general, the major toxic exposure is associated with a large number of low-dose compounds and the interactions between these compounds. This 'cocktail effect' is why the presence of even very small amounts of some specific pesticides can perturb an organism's hormonal balance (13, 14). Indeed, some pesticides bind as agonists to hormone receptors, which results in direct cell damage and/or the dysregulation of one or more biochemical or genetic pathways. In turn, this dysregulation produces toxic metabolites and increases the level of oxidative stress (14), which affects both male and female fertility. There are more research data on the influence of various pesticides on male fertility than on female fertility. However, even the data on male fertility (with putative links between exposure and a range of disorders), especially those correlated with poor sperm quality and low testicular weight (15, 16, 17), make it hard to affirm the presence of a direct, causal effect in humans.

Compounds such as lindane, PCB, atrazine, and mancozeb might dysregulate hormonal status in women by decreasing LH concentrations and thus promoting oligo-ovulation/anovulation and follicle destruction (13, 14).

Pesticide exposure and female fertility

In 1994, De Cock *et al.* (18) investigated the impact of pesticide exposure in market gardeners in the Netherlands and found that the fecundability ratio fell as the intensity of pesticide exposure increased. In this study, 28% of couples in the 'high exposure' group and 8% of the couples in 'low exposure' group requested assisted reproductive technology (ART) support. Other studies have emphasized this effect of pesticide exposure in women, with lengthening of the time required to conceive (19, 20, 21, 22). A risk of spontaneous miscarriage has also been observed, particularly in Bretveld *et al.*'s (23) study of female farmers (odds ratio (95% CI) = 4.0 (1.14–14.01).

Data from the Agricultural Health Study cohort showed that menstrual cycles of women exposed to pesticides increased in length, although no distinction was made between occupational exposure and domestic exposure (24).

These results clearly converge on an impact of pesticide use (especially in an occupational setting) on female reproductive capacity and certain female infertility diseases, such as premature ovarian failure (POF), polycystic ovarian syndrome (PCOS), and endometriosis. Furthermore, pesticide exposure might have direct or indirect effects on oocyte/embryo quality and the clinical outcomes of IVF.

The association between pesticide exposure and certain female infertility diseases

The negative impact of pesticides has been investigated in animal experiments. Many deficiencies have been described, such as low ovarian weight (25, 26), impaired folliculogenesis (27), a high aneuploidy rate, and the acceleration of follicular atresia (25, 26, 27).

Indeed, women exposed to some endocrine-disrupting pesticides (such as atrazine, lindane, and maneb) have an elevated risk of long menstrual cycles or anovulation (13). Conversely, high blood levels of DDE (28) and DDT (29) are associated with luteal phase deficiency and short menstrual cycles. The most serious consequence is POF (Premature Ovarian failure), defined as the cessation

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of menstrual cycles before the age of 40 years and characterized by very low blood levels of anti-Müllerian hormone (AMH). The prevalence of POF is around 1%, as a result of genetic or autoimmune/metabolic factors or cancer therapy (1). Nonetheless, POF might sometimes be caused by environmental factors, including pesticide exposure. Indeed, a few studies have linked exposure to HCH, mirex (30), pyrethroids (31), and their metabolites (especially 3-phenoxybenzoic acid (32)), PCB, and DDT (33) to ovarian ageing and menopause at an earlier average age. Interestingly, low AMH levels were associated with the presence of DDT in the serum (detected in more than 40% of samples from patients with POF (33)) and with high concentrations of pyrethroids and their metabolites with 25–26% reduction (31, 32).

The presence of DDE, MXC, or simazine (a triazine herbicide) was associated with follicular atresia (34). Given that MXC, simazine, and other pesticides (as atrazine, endosulfan, and chlordecone) are oestrogen receptor agonists (in contrast to DDE, DDT, and vinclozolin), they may have oestrogenic and/or antiandrogenic effects (5, 10, 35, 36). This implicitly explains why some specific pesticides could be responsible for various female reproductive diseases to differing extents and why the effects of some pesticide compounds (such as DDE, PCB, and MXC) depend on the patient's genetic predisposition. Indeed, these pesticides were associated with abnormally high AMH levels and a high number of small antral follicles – reflecting the presence of PCOS (37, 38, 39). Polycystic ovarian syndrome is a heterogeneous condition that affects 5–10% of women. According to the Rotterdam diagnostic criteria for PCOS, two of the following three criteria must be met: infrequent or absent ovulation (oligospaniomenorrhea), an abnormal ovarian morphology on ultrasound, and hyperandrogenism (1).

Moreover, some pesticides (such as TCDD (40, 41, 42), PCB (43), and permethrin (44)) have a documented impact on the endometrium by increasing vascularization, cell proliferation, VEGF levels, and thus endometriosis (with prevalence of 0.8–6%) (1). However, other pesticides (cypermethrin (45), chlorpyrifos, malathion, diazinon, lindane, DDT, and pyrethroids (46)) have the opposite effect by indirectly inhibiting endometrial proliferation or causing oxidative damage to the uterus.

Many studies have found an association between pesticide concentrations in the follicular fluid on one hand and the number and quality of oocytes collected for IVF (47, 48), endometrial thickness, and embryo implantation rates (46) on the other. Studies of animal models have shown that pesticide exposure is associated with elevated oxidative stress, which may be responsible for the observed alterations (49).

Impact on oocyte quality

Exposure to pesticides might cause generalized oxidative stress (49), with the elevated production of free radicals and impacts on superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, and glutathione transferase. This might explain why oocyte quality is impacted; oocyte dysmorphisms may reflect poor competence for development into a good embryo for implantation.

Indeed, we have highlighted (50) a conclusive link between a high prevalence (>75%) of oocytes with centrally located cytoplasm granulation (CLCG) and the patients' exposure to pesticides in the highly agricultural Picardy area of northern France (annual pesticide consumption: ~3900 tons). As a result, ongoing pregnancy and live birth rates were lower in couples with a high prevalence of oocytes with CLCG than in couples with low (<25%) prevalence (14% vs 32% for the ongoing pregnancy rate and 13% vs 30% for the live birth rate, respectively). Conversely, the early miscarriage rate was higher (47%) in the high-prevalence group than in the low-prevalence group (11%; odds ratio: 3.1). This poor IVF outcome might be indirectly due to high levels of pesticide exposure (over 3000 g/ha), which engenders a higher risk of oocytes with CLCG.

In line with these results, some pesticides (e.g. atrazine, cypermethrine, DDT, dieldrine, MCX, and vinclozoline) affect folliculogenesis (via the granulosa and theca cells) and thus induce meiotic aberrations (aneuploidy) and follicular atresia (51). For example, DDT stimulates aromatase and acts in synergy with FSH to induce a premature rise in oestradiol levels, affecting oocyte maturation (51). Furthermore, PCB might damage the ovarian reserve, the follicular response to administered gonadotropins, and oocyte maturation (51). Indeed, exposure of immature porcine cumulus-oocyte complexes to an organochlorine mixture (48) or atrazine (52) during *in vitro* maturation was associated with an elevated incidence of incompletely matured oocytes. The researchers mentioned the following pathophysiological mechanisms: abnormal cellular shape (disrupted spindle morphology), abnormal mitochondrial activity, and alterations in oocyte DNA (48, 51, 52).

The pesticides most frequently linked to defective oocyte maturation and oocyte competence are malathion (53), parathion (54), MXC (55), DDD/DDE/DDT/PCB

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Table 1 Summary of the negative impact of various pesticides on oocytes, embryos, the endometrium, and clinical outcomes in IVF.

Pesticide		EReg	Oocyte quality	Oocyte maturation	Oocyte genetic	Embryo development	Endometrium	IR	CPR	References
Organophosphorus	Chlorpyrifos			+		+	+	+		(71) (46)
	Cypermethrin			+			+			(45)
	Malathion	+		+		+	+			(53) (46)
	Parathion	+	+	+	+					(80) (10) (54) (27)
Organochlorine	MXC	+	+	+	+					(55) (81) (49)
	TCDD						++	+	+	(40) (41) (42)
	DDD	++								(56)
	DDE	++	+	+		++	+			(28) (56) (39) (26)
	DDT	++	+	+	++	+	+	+	+	(29) (56) (31) (20) (47) (51)
	PCB	++	+			++	++	+++	++	(43) (56) (77) (78) (39)
	Dieldrin	+				++				(72) (58) (51)
	Endosulfan						++	+++		(71) (79)
	HCB	++						++	+	(57) (47)
	HCH	++	+	+					+	(30) (47)
	Lindane	+	++	++	+		+	+		(13) (59) (46)

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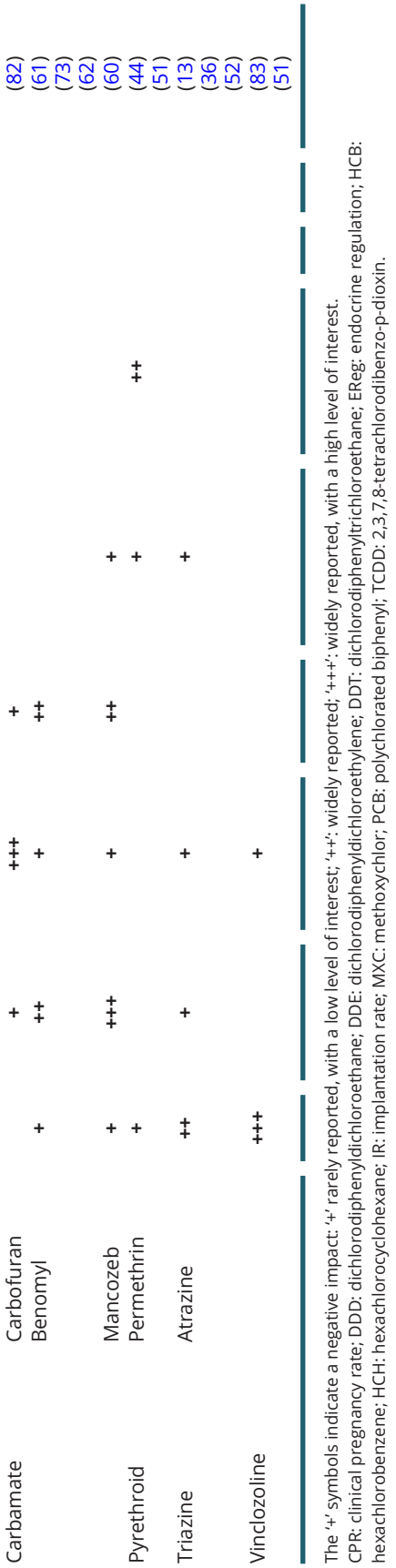

(56, 57), dieldrin (58), HCB/HCH (47), lindane (59), benomyl (60), and mancozeb (61, 62) (Table 1).

Interestingly, DDT was associated with a low proportion of diploid oocytes (56), while benomyl interfered with microtubules (60) and lindane was associated with cell damage (especially vacuolation and cytoplasmic fragmentation) (59). It was reported that mancozeb can strongly affect the meiotic spindle organization of oocytes (61), but the degree of damage could be decreased by treatment with resveratrol (62).

Moreover, exposure to deltamethrin (another common insecticide) in a mouse model was linked to cellular oxidative stress and meiotic abnormalities via DNA damage (63). Liu *et al.* (49) suggested that MXC exposure induces oxidative stress and affects mouse oocyte meiotic maturation via the accumulation of superoxide radicals and other reactive oxygen species (ROS), aberrant mitochondrial distribution, a low mitochondrial membrane potential, and elevated lipid peroxidation. Thus, exposure to MXC can negatively affect oocyte meiotic maturation – primarily through impairments in cellular metabolism. In general, all these pesticide-linked defects in oocyte quality are likely to degrade competence for the development of a genetically undamaged embryo.

Impact on embryo development

Pesticides can affect indirectly the embryo by dysregulating embryonic genome activation and embryonic metabolism, which is dependent on oxygen uptake. This latter is low at the 8-cell stage (64, 65, 66) and tends to increase after the morula stage required for blastocyst expansion (67). Blastocyst formation and the number of cells per blastocyst declined with the concentration of organochlorines (48) and atrazine (52). Furthermore, the association of pesticides like deltamethrin (63) and MXC (49) with poor metabolic and genetic status during embryo development might be due to the impairment of various biochemical pathways (13) and high ROS production. Moreover, high ROS concentrations and generalized oxidative stress are likely to affect the integrity of cellular constituents, such as DNA and proteins (68, 69, 70).

Various pesticides (especially chlorpyrifos with endosulfan (71), malathion (53), DDE/DDT/PCB (56, 47), dieldrin (72), mancozeb (73) (Table 1), and pretilachlor/diazinon (46)) may impact negatively embryonic development. Consequently, the use of low-quality embryos associated with pesticide exposure would give poor clinical outcomes (e.g. an elevated risk of miscarriage) in IVF programmes.

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Impact on clinical outcomes in IVF programmes

Women who consume high levels of pesticide residues in fruits and vegetables (74) or who live in an area with high pesticide exposure (50, 75) have an above-average risk of miscarriage. In one study, the probability of clinical pregnancy was 18% below average and the live birth rate was 26% below average in the women most exposed to pesticides (74). However, in a study in California, there were no differences in terms of spontaneous miscarriage, preeclampsia, and preterm birth rates between women exposed to pesticides (occupationally or through residence in an agricultural area) and unexposed women (76). Pesticides associated with poor clinical outcomes in IVF are chlorpyrifos (46), TCDD (42), DDT/PCB (46, 56, 77, 78), HCB/HCH (47, 56, 57), and endosulfan (79) (Table 1). Lindane, DDT, diazinon, and chlorpyrifos were associated with a low implantation rate but did not have a clear impact on the clinical pregnancy and live birth rates (46). Nevertheless, PCB and endosulfan have been linked to repeated implantation failures (77, 79).

Conclusion

A growing number of studies have assessed the putative causal link between exposure to pesticides and female fertility disorders. Although awareness of these issues has increased, the literature data are too scarce for conclusive, decisive recommendations. The impact of exposure to various endocrine-disrupting pesticides on fertility is now a public health issue that urgently requires the performance of more epidemiological studies – especially those focused on female fertility and women in IVF programmes. Furthermore, it is important to design studies that assess the severity of exposure and the nature of pesticides. There is also a need to develop more specific, rapid diagnosis techniques and treatments that might decrease pesticide-induced damage. Indeed, IVF participants living in agricultural regions should be informed about the fertility decline, low ongoing pregnancy rates, and elevated risk of miscarriage associated with exposure to high doses of pesticides.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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