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Environment Air Pollution Related to ART Facilities and Its Potential Implication with IVF Outcomes

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Abstract

Despite empirical experience and scientific evidence about how environmental pollutants are detrimental to reproduction and development, there are few studies correlating the presence of *in vitro* fertilization (IVF) known pollutants with deleterious effects over human embryos. Additionally, no centralization of official information on pollutants found within the (IVF) clinical setting is currently available. Relevant literature was reviewed on how these pollutants could impact the reproductive outcomes and the need of more research about the influence of pollutants, such as the Volatile Organic Compounds (VOCs) over the embryo development, is exposed. Types, sources, environmental control and some VOCs effects have been compiled for a better understanding of the evolution of the IVF's environment, and the path that remains ahead for waging against known and unknown pollution. Research results confirm that gametes and embryos in early stages of development can affected by VOCs, especially cells structures, interrupting cells communication, viability and changing their molecular profile, making them prone to develop hereditable mutations. There are adverse effects described on embryo maturation, morphology, cleavage, blastocyst development and implantation produced by air pollutants that lead to negative IVF and clinical outcomes.

Keywords: Pollutants; Volatile organic compounds; Particulate matter; IVF; Laboratory; Embryo quality

Introduction

The majority of Assisted Reproduction Technology (ART) facilities and its laboratories are located in urban areas where the levels of exposure to air pollutants are extremely high causing cardiopulmonary morbidity and mortality [1-4], as well as a host of reproductive health problems [5-9].

ART facilities are exposed to the pollutants produced in the external environment but, due to the daily routine activities and equipment within IVF laboratories, the air quality also diminish from the outside of a building throughout the laboratory [10-12]. In addition, although the incubators have controlled conditions, the environment provided is strongly influenced by unexpected sources of pollutants [13] as it was found that the highest values of toxic compounds were inside the IVF incubators because every time an incubator is opened, gas concentrations and temperature conditions can be significantly disturbed due to the large air-exchange volume with the laboratory´s ambient air (94-95%) or because the chemicals are released from the gas bottles (5%), especially if they have not received a routinely maintenance [14-16]. Similarly, a 5 to 6 fold increase of VOCs has been found inside the incubators and even higher in the IVF's adjacent areas [17]. And other authors have confirmed that the fertilization, cleavage and blastocyst formation rates increase after improving the air quality of the laboratory. However, certain compounds are capable of diffusing into the culture media and adversely affect gametes and embryos, at a sensitive stage, with devastating outcomes [13,18,19]. So guidelines have been developed to prevent these [17,20] but not much is yet known about all the pollutants that are detectable inside *in vitro* fertilization (IVF) laboratories and at which concentrations they represent a danger to embryos and future offspring, especially the VOCs.

Types of Pollutants: Particulate Matter and Volatile Organic Compounds

Atmospheric air pollutants can be categorized as primary pollutants (directly emitted from their sources into the atmosphere) or secondary pollutants (formed from photochemical reactions from primary pollutants), or classified according to chemical composition (organic or inorganic), sources (natural or anthropogenic), degradation properties (degradable or non-degradable), place of generation (indoor or outdoor), or based on the state of matter [21]. Additionally, the pollutants that are known or suspected to cause irreversible illnesses because of their toxicity, such as cancer or reproductive effects, are classified as Hazardous Air Pollutants (HAP) [2,22]. Inside the IVF laboratories, there are two general types of pollutants that take out attention: the particulate matter and the VOCs.

Particulate matter

PM is a complex mixture of extremely small solid and liquid particles (droplets) that can contain a wide range of inorganic and organic components [23]. These are the most common atmospheric pollutants and their mass and composition are strongly influenced by climatic and meteorological conditions. PM can be categorized as shown below (Table 1).

Most PM, especially the smallest fractions, are known to cause

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Table 1: Classification of particulate matter (PM) [23, 24].

Particles according to size	Origin
Inhalable coarse particles: larger than 2.5μm and smaller than 10μm.	Crust materials and fugitive dust found near roadways and dusty industries.
Fine particles or $PM_{2.5}$: 2.5μm or smaller and black carbon.	Aerosols formed from gas to particle conversion. Industries, automobiles, or forest fires.
$PM_{1.0}$: Less than 1.0μm.	Largest number of particles and the most hazardous in terms of mortality and cardiovascular and respiratory evidence.
Ultra-fine particles $PM_{0.1}$: Less than 0.1μm.	Nanoparticles: (Vaccines, personalized cancer therapy, drug delivery, and diagnostic methods)

Table 2: Classification of indoor VOCs [42- 44].

Type of VOCs	Examples of substance
Very Volatile Organic Compounds (VVOCs): Almost entirely as gases and difficult to measure.	Propane, butane, methyl chloride.
Volatile Organic Compounds (VOCs): Boiling point below 150°C	Limonene, toluene, acetone, ethanol, isopropyl alcohol, etc.
Semi-Volatile Organic Compounds (SVOCs): Higher boiling point and lower vapor pressure than VOCs. Highly related to $PM_{2.5}$.	A very wide range of individual substances including hydrocarbons, halocarbons, and oxygenates Present in both gas and particle phases in the air.

serious health problems due to their ability to penetrate deep into the blood stream and through different mechanisms or interactions that will depend on the type of exposition (acute and chronic, or outdoor or indoor exposure) [24-26]. The PM10 and PM2,5 are usually related to the outdoor ambient air, regular indoor spaces or occupational exposition. On the other hand, smaller particles are related to gaseous air pollution and to personal exposure [27]. According to the WHO, the most common PM associated with human health problems comprises the heavy metals [28,29], the Polycyclic Aromatic Hydrocarbons (PAHs) [30-34] or other organic components originated by the oxidation of VOCs, endotoxins and nanoparticles etc [27,35,36] .

Volatile organic compounds

VOCs are gaseous emissions of organic compounds, which have health implications such as reproductive toxicity [37]. These chemicals contain carbon (C) along with other elements (hydrogen, oxygen, fluorine, chlorine, bromine, sulfur, or nitrogen) and they are formed as intermediate compounds during the combustion, decomposition, or breakdown of longer-chain carbon compounds, as well as during the photosynthesis process in vegetation [38-40]. Additionally, they can volatize into the air from everyday products often under normal indoor atmospheric conditions of temperature and pressure given their low boiling point (less than or equal to 250°C at a standard atmospheric pressure of 101.3 kPa) [41]. The United States Environmental Protection Agency (EPA) has technically categorized these compounds depending on the ease with which they are emitted (Table 2) [42].

Sources of Indoor Pollutants

Outdoor pollution contributes to indoor air quality from type of ventilation (natural or forced), the ventilation rate (air changes per hour), and the nature of the contaminants [21,43-46]. On the other hand, PM and, specially, VOCs are present inside IVF laboratories through various vectors such as Heating, Ventilation, and Air Conditioning (HVAC) systems, diffusion of volatiles from adjacent rooms and hallways, off-gassing materials, equipment, people (perfumes and personal odors), medical and anesthetic gases, etc. Additional sources include potable water, dust, glass fragments, alcohol burners, disposable plastic ware and their shavings, markers, disinfectants, microscopes, television monitors, furniture, etc. Embryo toxicity risk in the clinical setting could in fact, be two to five times higher because VOCs are constantly released as different types of unsaturated volatiles and accumulate through the oxidation of air and light over routinely used materials, even when laboratories use appropriate materials to accomplish clean room standards to minimize pollutants [13,17,20,45,47]. Within 18 to 300 volatile compounds have been reported inside the laboratories, but it has not been done an official compilation on this matter on peer review and the majority of related investigations were carried out years ago [13,14,48-50]. The biggest concern is that VOCs, such as benzene, can be produced inside the incubators (CO_2 gas cylinders) and may contaminate gametes and embryos [10,51-54]. Additionally, VOCs are difficult to remove from IVF's ambient air and from the incubators, and they can interact with PM as well [13,47].

Control of Pollutants

The understanding on the infiltration and production of pollutants inside the laboratories and incubators is necessary to improve the design and preventive strategies to minimize contamination [47,55]. The first improvement concepts aimed at transforming other clean room designs, minimizing pollutants like vapors and particles accomplishing specific permitted values [52,53]. Nowadays, the quality control involves the improvement of the culture media formulations, contact supplies and gases used in IVF procedures [20,56] (Figure 1).

The isolation of the IVF lab, retrieval room, transfer room [57-59] and the design and adaptability of the laboratory to future improvements is essential [13,55,60,61]. Basic preventing strategies include: clean access for personnel and materials, double doors with windows for the anterooms between the Operating Room (OR) and the IVF lab to minimize the air mixing, a separated laboratory with a safety fume hood to use fixatives and toxic reagents and a separate area for cleaning and sterilization of materials. On the inside, the air management can be achieved through laminar flow cabinets, positive pressure and air filtration systems considering that dust particles of <0.5um in diameter often carry bacteria and/or fungi as well [10,20,57-59]. There are different filtration systems such as High Efficiency Particulate Air filtration systems (HEPA) which removes particles larger than approximately 0.3μm [51,62,63]. The Ultra Low Penetration Air (ULPA) [57,59], activated carbon filters, potassium-permanganate filters [19,63,64], photo-catalytic units [52], UV radiation [65], and filtration units within the incubators,

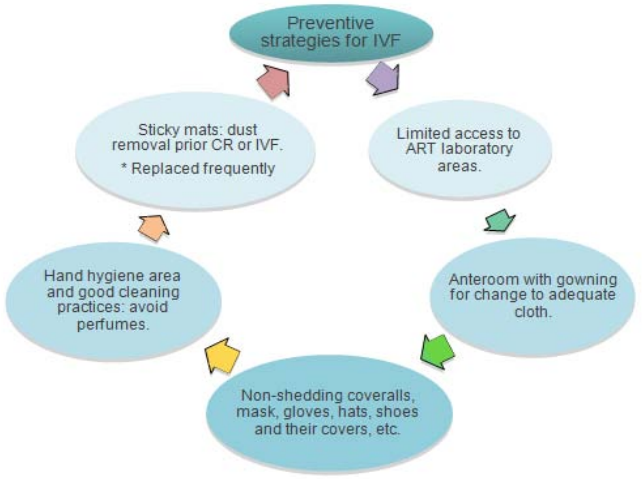

Figure 1: The quality control involves the improvement of the culture media formulations, contact supplies and gases used in IVF procedures.

chambers and filters in the incoming gas lines (CODA) [13,66,67]. Activated carbon absorbs higher molecular weight hydrocarbons (PAHs) through pores of varying size and a field of molecular attraction that captures large flat electron-rich molecules. Low molecular weight organics, alcohols, ketones and aldehydes can be oxidized and degraded by potassium permanganate. Photo Catalytic Oxidation (PCO) technologies are also used to filter VOCs [19,68]. New proposals in IVF isolation, engineered molecular media and genomically modeled biological inactivation are also in development and have shown significant increase in blastocyst conversion rates [55,60].

As to control the IVF pollutants, various parameters could be measured, such as; compound concentration and composition, solubility and vapor pressure (specially for VOCs), particle size, shape, surface modification and degree of agglomeration, as well as the ambient temperature and the surface area from which they could be released [53,68,69]. Specifically, the degree of solubility of compounds should be taken into account because they might penetrate the mineral oil layer and pass into the culture medium. The molecules penetration can be estimated by partition coefficients from air to oil (with vegetable oil) and oil to water (with octanol) to determine if it is soluble in both oil and water. The latter technique has been used to evaluate the risk of absorption of several compounds into the culture media; negative values of the octanol-water partition coefficients indicate that a compound is most likely to be absorbed by the media (eg: acrolein, -0.01) [49,53].

It has been reported that despite passing the manufacturers bioassays, mineral oils have affected embryo development, meaning that there is a lack of sensitivity of the tests. Nonetheless, the use of a good quality mineral oil can be achieved by washing it [70,71] and likewise, by improving the toxins screening through the improvement of the bioassays (Human Sperm Motility Assays (HSMA) + 1-cell Mouse Embryo Assays (MEA) or MEA with time-lapse) [71-74]. Recently, it was described a modification that could be more suitable for many laboratories: the extended MEA (eMEA) is more simple and sensitive when assessing the cells number and blastocyst formation rate was at 144h (instead of the 96h assessment as the regular technique) of individually cultured embryos, because group culture can stabilize the embryo environment and mask toxicity [75].

The specific requirements for IVF laboratories are different due to variations in regulations among regions [76]. Environmental and work health institutions (WHO), Occupational Safety and Health Administration (OSHA) and the "Instituto Nacional de Seguridad e Higiene en el Trabajo" (INSHT) in Spain, have chemical standards for evaluating industrial hygiene and health. These were only designed to cover workers that could be exposed every day without adverse effects, but they are not designed for cultured and largely unprotected cells such as the embryos and gametes as they lack physical barriers (epithelial surfaces), immunological defense or detoxifying mechanisms [51,53,68,77,78]. For general contamination, threshold limit values can be obtained and registered in concentrations of milligrams (mg/m^3), parts per million (ppm), or micromoles (me). To measure and evaluate the negative effects in cultured cells, limit values need to be in much lower concentrations ($\mu g/m^3$, ppm, or ppb). Unfortunately, specific quality standards and specific threshold levels at which contaminants cause harm to embryos have not been determined [53]. Further, the measured composition of air pollutants, such as VOCs, can vary significantly depending on the methods and recognized terminology, lending to confusion [41].

Effect of Pollutants on IVF Outcomes

The reported pollutants effects have been primarily related to acute and chronic cardiopulmonary affections through the activation of local and systemic inflammatory pathways, which promote systemic oxidative stress and inflammatory responses, thrombosis and coagulation, vascular dysfunction [79,80], epigenetic changes and genotoxicity (suppression of DNA repair and more DNA errors) [80,81].

However, the adverse effects over human reproduction can vary widely and are not entirely understood; hypotheses about how pollutants affect the embryonic development are still weak because they are based on limited data [17,49,51,57]. Studies and reviews had focused mainly in the relationship between pollutants and birth defects [82], or Low Birth Weight (LBW) and preterm births (almost 60% of LBW) which have been related mostly to pregnant women exposed during their 1st trimester [83-86], lack of fetal immune development [87] or menstrual disorders and their possible relationship with higher incidence rates of spontaneous abortion [88] etc. that can also lead to intrauterine and infant mortality [66,89,90]. But less is known about sub-fertile patients undergoing reproductive treatments which gametes and embryos are more susceptible to environmental influences because they lack of the physiological maturity of a differentiated mammal to protect themselves [5,17,51,91].

The IVF filtration systems have change the organic chemistry of the laboratories and incubators ambient air, improving embryo development, IR, PR and other outcomes [5,19,57,59,63,92]. For example, it has been reported an accelerated progression of development from early embryos up to blastocysts stage, a higher proportion of expanded and hatched blastocysts and a significantly higher number of blastomeres when cultured inside an enclosed system that protect oocytes and embryos throughout the IVF process [55]. However, some pollutants are still difficult to eradicate; so

detailed information about the relationship between pollution and developmental parameters (morphology, cleavage rate, symmetry, fragmentation, multi-nucleation, embryo development rate inside incubators, hatching process and defense mechanisms) require more attention [5,53,63,93]. For this reason, it is necessary to understand the toxic mechanisms over the development embryo because the pattern of substance distribution and action varies with each compound being related to the molecular weight, solubility, and degree of ionization at a physiological *p*H [18]. One of the main mechanisms related to exposure to air pollution (mainly studied for cardiopulmonary diseases) is the oxidative stress and few studies have related this in early human development with clinical outcomes in pregnant women [94]. Oxidative damage produced by pollutants has showed time-dependent cumulative effects and it can affect the membranes potential of mitochondria or produces apoptosis. Embryonic stem cells have shown different responses compared to somatic cells when exposed to pollutants or antioxidant treatments [95]. Oxidative stress-related genes and pancreatic and eye-lens gene markers appear de-regulated in embryos exposed to urban pollution, whereas exposure to rural extracts affected genes implicated in basic cellular functions [34,81].

Effects of particulate matter on the embryo development

Specific effects of airborne PM have been described mainly in animal models. It has been observed a significant impairment in fertilization, zygotes, embryo development, lineage of specification in blastocysts (ICM, TE and cell count), hatching, survival and post implantation potential after exposition to PM2.5 [93] heavy metals [93,95-99], nanoparticles [35,69,100,101] or PAHs [30,102,103].

PM2.5, PM10, Nitrogen dioxide (NO_2) can interfere with the IVF process, after chronic or acute exposure during the follicular growth phase [5,91,104,105]. There can be harmful effects on conception and intrauterine pregnancy due to increasing PM2.5 levels throughout the period from retrieval to transfer. In the presence of NO_2, mainly in the period from embryo transfer to pregnancy confirmation, live birth rates are affected [5]. Carré et al., reported lower number of top embryos and decreased implantation rates after acute more than after a chronic exposure to NO_2 [5,105].

PM may have influence over specific mechanisms; it could affect the Zonula Occludens (ZO-1), a protein that regulates tight junction formation between cells. This protein is first expressed during the compaction of eight-cell mouse embryos and it has been suggested as a necessary mechanism for blastocyst formation, helping in the differentiation of the Trophectoderm (TE) and ICM. When its function is altered, the number of formed blastocysts can decrease significantly and produce degeneration as it affects the number of embryonic cells, the bi-functional barrier that limits the diffusion of solutes, and the epithelial cell polarity [106]. Although this study was not correlated with a specific substance, a follow up study found negative effects associated with the interaction between different types of PM and pulmonary cells; the protein their degradation was evident as proteins were relocated from the cell periphery, disrupting the epithelial barrier [107].

Effects of volatile organic compounds on embryo development

In routine ART laboratory audits, it is recommended to evaluate the VOCs concentrations in the air among other pollutants, to prevent occupational hazards; however, while these substances do not often surpass the OLV due to current environment management strategies, there is still no certainty on how they interact with gametes or embryos during culture. A seasonal influence of the VOCs over the IVF laboratory`s air has been found to be related to the outside temperature and humidity and over the embryo development and implantation rates [108,109].

The first lethal effects by IVF-VOCs emissions were reported in early mouse embryos [17,51]. Later, the VOCs-specific filtration systems have been correlated to higher rates of fertilization, cleavage, blastocyst development and higher embryo fragmentation as a possible mechanism for the embryos to improve their developmental competency and in consequence, a reduction in spontaneous abortion, improved implantation and pregnancy rates [13,62,66,67].

Limonene it is a well-known VOC because it is used as an additive in cleaning products and it is very common to find it inside of the IVF laboratory. Despite of this, no studies have been performed evaluating possible early life deleterious effects because it is known as a low toxicity VOC and it is considered that it does not have strong mutagenic, carcinogenic or nephrotoxic effects. However, a couple of studies have reported that it does induce a few cell transformations in Syrian hamster embryo cells [110], or that influences mechanisms of increase intracellular Ca^{++} pathways and Ca^{++} activated potassium (BKCa) channels, which could be correlated to increasing myometrium contractions [111]. Trichloroethylene (TCE), is a highly volatile inhalation anesthetic used mainly in short surgical procedures. Significant anomalies in skeletal and soft tissues, indicative of delay in the development, have been observed in groups exposed to TCE during pregnancy in rats [112]. TCE embryonic genotoxicity effects have been described causing cardiac valvular and septal malformations or it can disrupts calcium (Ca^{**}) flux regulation in embryonic myocites [113-116]. TCE may alter the permeability of the cell membrane causing an electrolyte imbalance. TCE caused great changes in gene expression during critical phases of the heart development [115]. Acrolein is an airborne VOC that has been directly related to negative effects on cleavage, cell number, and blastocyst development [17,117]. However, a recent study demonstrated that its negative effects (embryos arrest at 1-cell to morula stage over the embryo development can be diminish through a proper protein concentration in the medium and the quality of the oil [118]. Toluene has a high affinity for lipid-rich tissues. Significant degenerative changes have been found in preimplantation embryos exposed to toluene *in vitro*: decreased fertilization rate of exposed oocites and embryo degeneration resulting in increased embryonic lethality [119]. In animal models morphological anomalies and congenital defects in early female fetuses (gestational D8 to D20) have been found [120-122]. It has been described also that toluene cytotoxicity effects on human embryonic stem cells are comparable to 1-octen-3-ol and its enantiomers (a major fungal VOC associated to indoor mold and odors) [123]. When exposing murine bone marrow stem cells to two fungal VOCs [(E)-2-octenal and oct-1-en-3-ol], a shift to unsaturated fatty acids and lower cholesterol levels in the cells membrane was produced, which means increased the membrane fluidity, and this could be related to malfunction of the immune system [124].

Benzene (C_6H_6) is a compound that is considered very harmful in general. It is also frequently found inside laboratories. There are no threshold levels for this substance inside laboratories and there is little evidence linking benzene to IVF reproductive outcomes. Meiotic delay of MI oocytes and frequencies of aneuploidies in MII mouse oocytes were observed after a dose-dependent inhalation of benzene, especially with higher doses in a "multiple inhalations" group [125]. Tsutsui et al. demonstrated a marked dose-dependent genotoxicity on Syrian hamster embryo cells when exposed to benzene and its metabolites. Some of the effects seen were disturbed cell growth, cells transformation, increased frequency of chromosomal aberrations (gaps and breaks), alterations in chromosome numbers, and genetic mutations. Catechol is the most harmful metabolite for cells at lower concentrations, but hydroquinone and phenol also have negative effects [126]. Benzene or metabolites mixtures (catechol, hydroquinone and benzoquinone) produce diverse effects in mouse cells from individuals of different ages and genders as well: 16-day-old male and female, adult males, females, and pregnant females [127]. In utero exposure studies have suggested predisposition of the embryo or fetal tissues to carcinogenesis as well, caused by alterations in the redox signaling pathways, excess of production of Reactive Oxygen Species (ROS) and therefore oxidative stress, affecting the regulation of gene expression, cell growth, and cell death. But there are a lot of intrinsic differences in the susceptibility of the target cells (type, age, gender) as well and it could critically alter cell-signaling pathways necessary for normal hematopoiesis. Male fetuses have been found to be more susceptible to benzene-induced ROS production after two hours of exposure. Benzene was observed to be very deleterious for unprotected embryos given their rapid growth and developmental changes depending on the cellular signaling that occurs during embryonic development [128,129]. Benzene levels have been significantly related to a positively trend in baseline FSH levels and negatively trend of E_2 peak levels, average number of oocytes retrieved and average number of embryos transferred. The intra-ovarian levels of benzene were associated with hypo-sensitivity of follicles to endogenous and exogenous gonadotropin, leading to an unknown mechanism of resistance [130].

Conclusions

Changes in IVF laboratory air quality have been crucial in influencing conception rates, embryonic development, implantation, and live birth rates in human reproduction. The *in vitro* environment must be monitored and improved by far more than just the culture media formulations and conditions due to the increased risk of exposure to different pollutant compounds inside the incubators and during routine handling inside in the laboratory of the gametes and embryos.

Despite the fact that IVF clinics perform routine environmental audits and have developed preventive strategies, there remains a high degree of uncertainty about reproductive health effects and even more on how pollutants can interact with embryos. Environmental monitoring of laboratories and surrounding spaces will continue to be insufficient until the harmful pollutant values can be standardized into official embryo-toxicity thresholds. So far, the data gathered has progressively allowed to research about specific warning parameters warning of possible embryonic hazards, but so far, the repro-toxicologic studies with specific effects reported on different stages of human embryo development *in vitro*, are very limited. Most have been performed in other animals and these results are not completely translatable to humans due to the differences in species or type of culture (medium and oil overlay) or just by the differences of pollutants, doses and studies design.

Further investigations are needed to develop sensitive and relevant quality control assays for culture system as well [73]. The absorption mechanisms of each substance should also be considered due to the variability of their physical and chemical characteristics, most specifically solubility parameters, which may determine the brevity in which the exposure will result in developmental failure and long-term negative effects on fetuses.

Specific morphologic parameters of the gametes, zygotes, and embryos should be assessed systematically when evaluating air quality due to evidence that pollutants can affect development at the earliest stages (the four cell stage and compaction up to the differentiation of the ICM and TE), which have fundamental roles in embryo survival, implantation, and fetal viability. However, since embryos often develop even in the presence of contaminants, other molecular parameters such as chromosomal abnormalities or epigenetic modifications should be considered because the epigenetic biomonitoring is necessary and it needs an international methodological accordance [131]. It would be remarkable to ascertain the role of embryonic self-defense and repair mechanisms against pollutants, such as the mechanisms of fragmentation and abortion, as well as long-term effects on children conceived through IVF techniques.

Finally, because all the studies reviewed originate from different sources, study designs and findings varied, it is therefore important to consider that future studies should have similar designs so that results can be easily interrelated and associated with previous findings so as to establish specific developmental rates after exposure and perhaps develop an official database with IVF known pollutants and their effects.

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