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ENDOMETRIOSIS (P STRATTON, SECTION EDITOR)

The Role of Endocrine Disruptors in the Epigenetics of Reproductive Disease and Dysfunction: Potential Relevance to Humans

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Abstract In a murine model, we have linked early life toxicant exposure to reduced uterine sensitivity to progesterone, a phenotype we had previously associated with inflammation in endometriosis patients. Subsequent studies revealed that developmental toxicant exposure not only reduces fertility in male and female mice but also negatively impacts pregnancy leading to spontaneous preterm birth (PTB), regardless of which parent was exposed. An epigenetic alteration of the progesterone receptor gene correlated with reduced fertility and adverse pregnancy outcomes and persisted in multiple generations of mice in the absence of an additional toxicant exposure. Gene–environment interactions in women may explain why some patients "at risk" for PTB deliver at term while others without known risks deliver early. Our model provides a unique system to unravel the interactive influences of inflammation and reduced progesterone responsiveness on PTB. Our model suggests that therapy needs to begin prior to conception (and involve both partners) rather than during pregnancy once the inflammatory cascade has been initiated.

Keywords Endometriosis · Preterm birth · Progesterone · Inflammation · Epigenetics · Environment · TCDD

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Introduction

The primary biological role of the human uterus is to provide a supportive in vivo environment for optimum fetal growth and development before live birth. Despite this singular purpose, coordination of the initiation and maintenance of pregnancy within the uterus requires a complex interplay between the maternal endocrine and immune systems, the paternally derived placenta, and the fetus itself. Cellular communication missteps at the interface of these two systems within the uterus can result in infertility, pregnancy loss, or preterm birth (PTB). A significant impediment to therapeutically improving fertility and avoiding adverse pregnancy outcomes is our imperfect understanding of key elements of uterine function during each interval of human gestation as a consequence of ethical considerations that limit direct studies. Nevertheless, it is well known that the establishment and maintenance of pregnancy are progesterone-dependent processes; thus, defects in the production or action of this steroid, as a result of environmental stressors or an individual's genetic or epigenetic predisposition, can undermine pregnancy success. Additionally, a growing body of evidence suggests that reproductive tract diseases and dysfunction may be a consequence of a previous exposure to bioactive chemical contaminants capable of inducing epigenetic alterations [1–4, 5•]. The potential that epigenetic alterations in either the male or female germ line may continue to negatively affect reproductive health for multiple generations suggests an urgent need to better understand the mechanisms and functional impact of toxicant-mediated cellular changes so that appropriate therapeutic intervention strategies can be developed. To address this challenge, our translational research group has explored the mechanisms by which TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) may negatively influence progesterone action related

to the development of endometriosis, a common reproductive disorder affecting fertility and pregnancy outcomes in women worldwide.

Endometriosis, the presence of endometrial glands and stroma outside the uterus, remains one of the most poorly understood conditions affecting not only women's reproductive potential, but also their overall quality of life. Significantly, the human endometrium is an important component of the mucosal immune system; thus, coordination between the endocrine and immune systems is critical for successful pregnancy. Consequently, disruptions in cell–cell communication affecting the interface of the endocrine and immune systems within the reproductive tract are likely sentinel, inflammation-related events in the development of endometriosis and other reproductive disorders. Specifically, we have shown that the loss of endometrial progesterone sensitivity, a well-recognized component of endometriosis, is biologically linked to an inflammatory-like pattern of cell–cell communication within the uterus [6–8]. Importantly, altered patterns of cell–cell communication likely impact other areas of the reproductive tract in women with endometriosis, potentially compromising pregnancy success [9]. Whether reduced reproductive tract responsiveness to progesterone leads to the development of endometriosis or emerges as a consequence of the inflammatory nature of the disease is currently unknown. Nevertheless, once in place, a loss in the differentiation-promoting, anti-inflammatory actions of progesterone would be expected to functionally compromise numerous aspects of reproductive success [10]. While reduced fertility has long been associated with endometriosis (see [11] for review), the anti-inflammatory action of progesterone is equally critical for the *maintenance* of pregnancy; thus, it is not surprising that fertile women with this disease also possess an increased risk of delivering preterm [12].

Environmental Endocrine Disruptors in Reproduction

"What are the trigger mechanisms leading to progesterone resistance in the adult reproductive tract?" and "when are these triggers activated?" are critical and currently unresolved questions related to environmental disruption of progesterone-mediated reproductive success. For example, in co-cultures of stromal and epithelial cells acquired from the endometrium of disease-free adult donors, we have shown that short-term exposure to TCDD triggers an inflammatory-like pattern of cell–cell communication that reduces the stromal cell expression of the progesterone receptor-B (PR-B) isotype [13]. In turn, blocking this inflammatory-like pattern of stromal-epithelial communication in similarly treated human endometrial cultures prevented the TCDD-mediated loss of PR expression [14]. However, our capacity to detoxify TCDD and other toxicants as adults is much greater than our early life capacity to limit the impact of similar exposures [15]. Human exposure to environmental toxicants begins in utero and continues throughout our adult reproductive lives, and some chemically stable, fat-soluble toxicants accumulate in our bodies. As will be discussed, a transfer of the potentially negative influence of environmental toxicants can occur indirectly via epigenetic alterations, which can be propagated across multiple generations [1, 4, 5•]. Thus, it is important to ascertain the relative risk of environmental toxicant exposures across an individual's lifetime to understand the origin(s) of the reduced progesterone sensitivity observed in the reproductive tract of endometriosis patients.

Numerous chemicals, both natural and manufactured, may act as disruptors of endocrine and immune system development during critical periods of gene programming, not only potentially compromising adult reproductive health [16] but also promoting cancer and diabetes-related obesity [17]. It is estimated that more than 80,000 chemicals have been released into our environment within the United States over the past few decades, and current regulations do not require prospective risk assessment of these multiple, potentially interactive chemicals on human health [18]. Thus, a significant challenge to the study of environmental toxicants that may disrupt human reproductive health is the initial choice of an individual toxicant for study. Our choice of TCDD as a prototypical endocrine-/immune-disrupting toxicant for our studies reflects the well-established toxicity and high affinity binding characteristics of this compound to the aryl hydrocarbon receptor (AhR). The AhR, an orphan nuclear receptor, is abundantly expressed within human and murine reproductive tissues [19, 20]. A number of environmental endocrine disruptors produced by industrial processes and/or present in cigarette smoke bind this receptor and disrupt reproductive processes across multiple species [21].

TCDD and other polychlorinated dibenzo-p-dioxins, generally called dioxins, are members of a family of chlorinated aromatic hydrocarbons that accumulate as ubiquitous contaminants in our environment; thus, ingestion of contaminated food is the primary source of human exposure [22–24]. TCDD is considered to be the most toxic dioxin congener and has been shown to disrupt steroid receptor levels, metabolism, and transport [25–27]. Significantly, TCDD and dioxin-like polychlorinated biphenyls (PCBs) readily cross the placenta and accumulate in breast tissue, allowing exposure of the fetus during pregnancy and the neonate during lactation [28]. Within our human and rodent toxicology studies, we utilized TCDD as a research tool to specifically define critical elements of steroid-mediated cell and tissue function within the reproductive tract that may be susceptible to disruption by environmental toxicants with dioxin-like action. Because progesterone plays an absolutely critical role in stabilizing the

maternal–fetal interface in support of pregnancy, we focused our rodent work on the ability of TCDD to disrupt the anti-inflammatory action of progesterone during gestation. As discussed below, we have linked developmental toxicant exposure of female mice to reduced fertility and an elevated risk of PTB for multiple generations [4], while a similar toxicant exposure of male mice also leads to adverse reproductive outcomes [29, 30•]. Given the findings of our rodent model and others as noted above, it would be prudent to consider the possibility that early life environmental exposures in women might lead to epigenetic modifications that negatively affect adult reproductive tract function.

Basic Epigenetic Mechanisms

For over half a century, human genetics research has focused on DNA as the heritable molecule responsible for carrying phenotypic information from the parent to the offspring. According to this paradigm, changes in phenotype would underlie changes in DNA sequence due to mutations in single genes or a small number of genes. However, epigenetic marking of DNA is now recognized as an important mechanism by which gene expression can be altered without a change to the DNA sequence. Although these marks are not necessarily permanent, they are stable and inheritable [31, 32], and thus, in addition to genetic mutations, epigenetic modifications also contribute to an individual's overall appearance, reproductive capacity, and susceptibility to disease. During development, many cells undergo reprogramming, in which epigenetic marks are removed and new ones acquired, resulting in cellular differentiation along a particular path within each organ system despite the presence of identical DNA. In this manner, epigenetic modifications determine a cell's destiny and the unique phenotypic and functional characteristics of specific tissues and organs. Within the differentiated somatic cells of adults, epigenetic modifications accumulate as we age, and are in large part the reason we "get old" and become more susceptible to certain (age-related) diseases. Nevertheless, although epigenetic marks can be acquired over an individual's lifetime, these changes do not usually affect the phenotype of the next generation. In contrast, environmental factors that affect epigenetic marks within the germ line can be inherited and have the potential to contribute to an offspring's phenotype [33].

The primary role of epigenetic modification is to control DNA accessibility (ie, the ability of the cellular machinery to switch genes on), thereby activating transcription. The best-described mechanisms of epigenetic changes are methylation and acetylation of DNA and histones [34]. Hypermethylation of DNA is generally associated with gene silencing, while hyperacetylation of histones leads to chromatin relaxation and unwinding, which promotes gene transcription. DNA can be methylated at cytosine residues, frequently within the promoter regions of specific genes, but also within distal regions of DNA that together contribute to epigenetic regulation [35, 36]. DNA methylation can downregulate gene transcription by physically impeding the binding of transcription factors to the promoter or by enhancing the binding of methyl-CpG–binding domain proteins (MBDs) to DNA, which leads to the formation of compact, inactive heterochromatin. Epigenetic modifications can also target histones, which impact DNA accessibility. At the chromatin level, histones can be covalently modified via acetylation, phosphorylation, methylation, ubiquitination, sumoylation, or adenosine triphosphate ribosylation or combinations of these mechanisms. Hyperacetylation of chromatin proteins leads to open DNA and transcriptional activation while histone deacetylation leads to gene silencing. Residing in the genome of normal cells within various organ systems there are "healthy" patterns of methylation and acetylation, which appropriately regulate the transcription of certain genes. Not surprisingly, the presence of aberrant epigenetic patterns can cause developmental abnormalities and are associated with the etiology of human diseases [37] in addition to the reproductive failures discussed herein.

Thus, in addition to information present within an individual's specific genetic code (DNA sequence), epigenetic alterations can modulate how this otherwise hardwired genetic information is utilized within a cell. Significantly, although such epigenetic changes are stable, they are themselves also subject to modification. Specifically, while lifestyle choices such as smoking can promote epigenetic changes that effectively accelerate aging, epigenetic therapies that can reverse these changes in order to combat certain cancers and other diseases are also being designed [38–40]. For most couples, developing lifestyle choices that preserve a healthy epigenome not only may dramatically reduce their individual disease susceptibility but also may promote the long-term health of their future offspring [41–43].

Evidence for Early Life Origins of Reproductive Dysfunction in a Mouse Model

As noted above, the environmental endocrine disruptor TCDD was selected as a "prototypical toxicant" for our reproductive toxicology studies based on several rodent, primate, and human studies that associated chemicals with dioxin-like action to the development of endometriosis (reviewed by [6]). Our initial studies utilized human endometrial cell culture models for analysis of cellular responses to acute TCDD exposure and an in vivo chimeric model of human experimental endometriosis in immunocompromised mice. This dual approach allowed us to demonstrate that

acute, in vitro TCDD exposure was associated with the development of a progesterone-resistant endometrial cell phenotype, which promoted establishment of an endometriotic-like experimental disease [13, 44]. We additionally demonstrated that acute TCDD exposure of human endometrial tissues disrupted the anti-inflammatory action of progesterone and led to an increase in endometrial tissue sensitivity to inflammatory cytokines, a toxicant-mediated response that increased the secretion of matrix metalloproteinases (MMPs) and promoted the invasive potential of endometrial tissue in our chimeric experimental endometriosis model (reviewed by [44, 45]).

The noted laboratory-based studies and similar studies using adult cells and tissues suggest that TCDD-mediated activation of the endometrial MMP system may represent a key mechanistic pathway linking toxicant exposure to the invasive establishment of endometriosis [6]. However, as opposed to experimental evidence, epidemiologic studies attempting to associate endometriosis with adult body burdens of dioxin-like environmental toxicants in various human populations remain inconclusive (reviewed by [6, 46]). Perhaps explaining this discrepancy, humans and other mammals are most sensitive to environmental toxicant exposure during their development, and epidemiology studies attempting to link adult body burdens to a current disease status does not adequately consider the potential impact of early life exposures. Thus, in addition to human population studies, appropriate animals models are needed to examine the potential biochemical and functional effects of toxicant exposure during the in utero/early neonatal period that may negatively impact future reproductive outcomes. It is not possible to design an animal-based research strategy that duplicates complex human population exposure paradigms or the genetic variability among individuals. However, it is quite possible to utilize an early life toxicant exposure model in mice as a discovery tool to identify specific cellular and molecular signaling defects that may be equally involved in infertility and adverse pregnancy outcomes in humans. In particular, we designed our studies to explore whether an early life TCDD exposure would subsequently lead to an adult uterine phenotype that exhibited the reduced uterine progesterone response and heightened sensitivity to inflammation that our past in vitro studies had identified.

Within our studies, pregnant 10- to 12-week-old female C57BL/6 mice (F0, or founding generation) were exposed to TCDD (10 μg/kg) by gavage on embryonic day 15.5 (E15.5), when organogenesis is complete, similar to approaches used by previous investigators [47, 48]. This level of experimental TCDD exposure during late pregnancy *is not teratogenic or abortigenic,* reflects the rapid clearance of this toxicant in mice, and is well below the LD_{50} for this strain (230 μg/kg) [49]. Using this model, we reported that early life/in utero exposure of female mice to TCDD leads to an adult uterine phenotype in animals that mimics the reduced uterine progesterone responsiveness observed in women with endometriosis [50]. However, mating studies involving offspring from our exposure model revealed a reduced reproductive capacity in both male and female (F1) mice, with adult animals exhibiting a variety of reproductive phenotypes, similar to the human spectrum. Specifically, as shown in Table 1, within each litter, some male and female mice exhibit infertility at sexual maturity while other animals exhibit normal fertility when mated to an unexposed partner. Among mating pairs of mice achieving pregnancy, a relatively high rate of spontaneous PTB was also observed [4, 29]. Again, reflecting our in vitro findings following acute exposure of human endometrial cells and tissues to TCDD, the occurrence of PTB in toxicant-exposed F1 female mice occurred coincident with the phenotype of reduced uterine progesterone responsiveness and heightened sensitivity to inflammatory signals during pregnancy [4, 29]. An interesting and somewhat unexpected finding was that, among fertile F1 males, we documented a significant rate of PTB after matings to unexposed female partners concomitant with premature placental inflammation [29, 30•]. Furthermore, in the absence of additional toxicant exposures, diminished fertility and an increased risk of PTB was observed in *descendants* of either male or female F1 mice (Table 1).

Our findings of both infertility and PTB in our murine model of early life TCDD exposure is predictable since the F1-F3 female animals exhibit the same progesterone resistant phenotype as women with endometriosis [50]. Recent human studies in cohorts of women with endometriosis have suggested that these patients possess an increased risk for PTB [12, 51] as our murine model predicted [4, 6]. However, a novel aspect of our animal model, not previously recognized in most human studies, is that the early life/in

Table 1 Impact of developmental TCDD exposure on transgenerational reproductive outcomes of male and female mice

Mouse exposure history	Pregnancy rate[a]	Pregnancy outcome	
		Full-term	Preterm[b]
Vehicle control	15/15 (100 %)	15/15	0/15
TCDD in utero			
F1 female	15/36 (41 %)	60	40
F3 female	10/17 (61 %)	63	37
TCDD in utero			
F1 male	27/58 (46.5 %)	67	33
F3 male	9/20 (45 %)	78	22

TCDD 2,3,7,8-tetrachlorodibenzo-p-dioxin

[a] Most animals required multiple mating attempts to achieve pregnancy. Mice which did not achieve pregnancy following 4 positive vaginal plugs were considered infertile

[b] Preterm was defined as spontaneous parturition 24 hrs prior to expected term delivery on E20

utero toxicant exposure history of *either* parent to TCDD may impact PTB by disrupting progesterone-mediated patterns of cell–cell communication at the maternal–fetal interface [29]. In this regard, a central finding of each of our human and murine studies to date is that the ability of TCDD exposure to disrupt the anti-inflammatory action of progesterone and subsequently *increase the reactivity of cells and tissues within the reproductive tract to proinflammatory signals*. The importance of this central observation to reproductive success emerged when we observed a doubling of the PTB rate in response to infection-related inflammation during a pregnancy study in F1 females. Following an unexpected parvovirus (MPV) outbreak in our colony, 86 % of pregnant female F1 mice and 67 % of F3 female mice delivered preterm compared to 37 % and 25 %, respectively, in similar studies conducted in the absence of MPV [4]. Importantly, pregnant control mice housed within the same colony never delivered preterm, even during the MPV outbreak [4]. This serendipitous finding suggests that the *TCDD-mediated decrease in progesterone responsiveness may have only a modest impact on length of pregnancy in the absence of an additional stressor.* To confirm the dual-hit etiology for increased PTB in our model, we conducted a follow-up study using a very low dose of LPS (lipopolysaccharide; 200 μg/kg), which does not induce PTB in control animals of the same strain. As predicted, while none of our control mice exhibited PTB following exposure to our low-dose LPS paradigm, PTB was observed in 100 % of female mice with a history of direct (F1) or ancestral (F3) exposure to TCDD (Table 2). While a similar human study would not be possible, our murine study clearly indicates that increased sensitivity to inflammation is one mechanism by which early life toxicant exposure may lead to an increased risk of PTB at sexual maturity.

Our data, demonstrating reduced fertility in both sexes and increased risks of PTB in female mice across multiple generations following a single, developmental exposure to TCDD strongly suggests toxicant-mediated epigenetic modifications have occurred. To more closely examine the potential epigenetic consequence of developmental TCDD exposure, we examined the methylation status of the progesterone receptor (PR) gene in uterine samples from our female mice. As previously discussed, DNA hypermethylation can mediate gene silencing and is an important mechanism by which inheritable epigenetic modifications may occur [33]. Our in vitro toxicant exposure studies demonstrated that TCDD-associated suppression of PR-B gene and protein expression may be mediated by the local action of toxicant-induced inflammatory cytokines [13, 14]. Additionally, Wu et al. [52] demonstrated that treatment of isolated human endometrial epithelial cells with tumor necrosis factor–α led to hypermethylation of the PR promoter and a loss of PR-B protein expression. Taken together, these in vitro human cell data suggested to us that the loss of progesterone sensitivity associated with developmental exposure of mice to TCDD may be due to an epigenetic modification, mediated by inflammatory processes. As shown in Fig. 1, examination of murine uteri by methylation-specific polymerase chain reaction (MS-PCR) revealed partial methylation of the PR in 60 % of tissues removed from F1 females (exposed to TCDD in utero) while this gene was largely unmethylated in similar tissue samples acquired from control mice. To determine if PR gene hypermethylation is a transient or stable (inheritable) effect, we similarly examined tissues removed from F3 females (the "granddaughters" of F1 mice). As shown in Fig. 1, 40 % of F3 animals also exhibit partial methylation of the PR gene, suggesting that the transgenerational infertility phenotype and associated risk of PTB in these animals may be due

Table 2 Impact of low-dose LPS exposure in mice with a direct or ancestral history of TCDD exposure

Mouse history*	Time to delivery after LPS	
	≤16 h	At term
Control + PBS		100 %
Control + LPS		100 %
F1 female + LPS	100 %	
F3 female + LPS	100 %	

LPS lipopolysaccharide; *TCDD* 2,3,7,8-tetrachlorodibenzo-p-dioxin; *PBS phosphate-buffered saline*

* $n \geq 6$ per group

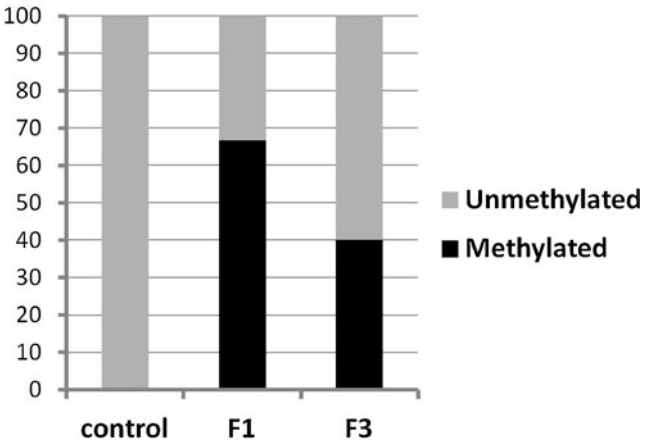

Fig. 1 Methylation-specific PCR of the progesterone receptor gene. Methylation-specific PCR of the progesterone receptor of whole uterine samples obtained from control, F1, or F3 female mice. Extracted DNA was subjected to bisulfite conversion and real-time PCR using methylation-specific primers and standard methodology [52] (n=5 for all groups; mice from multiple litters were used for each group). *PCR* polymerase chain reaction

to epigenetic modifications as a consequence of ancestral TCDD exposure.

Clinical Perspective

Our emerging appreciation that many diseases and conditions affecting adults are influenced by the prevailing in utero microenvironment [16] suggests that reproductive health also may be negatively influenced by disruption of normal fetal development. Within this review, we have discussed our previous human studies related to endometriosis as well as recent experimental findings in a murine model that demonstrates an epigenetic link between early life exposure to TCDD and the development of reduced progesterone sensitivity within the reproductive tract across multiple generations. Our studies also uncovered a novel role for male exposure to environmental toxicants on reproductive outcomes in their female partners. Clearly, prospective reproductive toxicology studies cannot be conducted in pregnant women or women seeking pregnancy and linking a couple's past environmental exposure histories affecting reproductive potential is difficult. Nevertheless, our murine studies suggest that couples may be "at risk" for both infertility and PTB as a consequence of the environmental exposures of their parents and grandparents. For example, within our animal colony, mice with direct or ancestral TCDD exposure exhibited a heightened sensitivity to exogenous inflammatory stimuli (MPV or LPS), which further affected the anti-inflammatory action of progesterone sensitivity and the related risk of PTB (Table 2). Animals without toxicant exposure never exhibited these phenotypic changes related to PTB despite also being exposed to MPV or LPS. Although intrauterine infections have been identified in only about one third of PTBs, sophisticated methodology has demonstrated the presence of bacteria in up to 70 % of women undergoing elective caesarean section at term [53]. These human data strongly suggest that the presence of bacteria alone is not sufficient to cause PTB in most women. Our results in mice suggest another necessary contributor to PTB might be a maladaptive inflammatory response within the reproductive tract related to changes in progesterone sensitivity. Thus, various stressors encountered during the course of most pregnancies (viruses, bacteria, or even emotional stress) may each represent a more significant risk factor for adverse outcomes in women with altered sensitivity to progesterone, perhaps amplified by a prior environmental toxicant exposure.

Certainly, the transgenerational studies presented within this review suggest that the toxicant exposure/adult disease relationship *can become disassociated not only across an individual's lifespan but across multiple generations.* This temporal dissociation suggests that a patient presenting with infertility or history of PTB may have no identifiable risk factors, yet she may still be at risk as a consequence of her ancestry or that of her partner. Given these confounders and the difficulty in obtaining an accurate exposure history within a clinical setting, a more logical approach may be to presume that most patients and their partners have a history of toxicant exposure (as a consequence of living in an industrialized country). In this regard, excess or inappropriate inflammation appears to be a recurring theme that contributes to the development of both reduced fertility and adverse pregnancy outcomes in our murine model. Thus, a strategy that assumes that a woman's individual sensitivity to various triggers of inflammation may be adversely affecting her reproductive outcomes could include dietary intervention in both the male and female partner using clinically acceptable therapies that would *do no harm* if the assumption of toxicant exposure is erroneous.

Omega-3 fatty acids are well-recognized as anti-inflammatory agents and have been suggested to reduce the risk of PTB in some but not all human studies [54, 55]. The finding of only a modest increase in gestational length may be due to the timing of current human therapy initiation (20 weeks and beyond) and the lack of consideration of the contribution of the male to adverse pregnancy outcomes. In our experimental model in mice, we have explored the impact of an omega-3 enriched diet in adult male animals with a known toxicant exposure history *before initiation of pregnancy* in an unexposed female partner. Our strategy was to introduce a *preconception* diet in male animals that would potentially reduce the inflammatory phenotype of the paternally derived placenta that we had previously associated with the risk of PTB [29]. Using this approach, we successfully prevented PTB in control female mice mated to F1 males provided a fish oil–supplemented diet for the 2 weeks preceding mating [30•]. At this juncture, we have not yet determined if the improved pregnancy outcomes associated with the supplemented diet was mediated by a change in the inflammatory epigenome of the placenta or simply a consequence of preserving the anti-inflammatory action of progesterone at the maternal–fetal interface. Nevertheless, a similar nutritional, anti-inflammatory approach in humans may be an appropriate clinical choice with regard to promoting fertility and protecting pregnancy without compromising the health of the patients and should be beneficial to the postnatal health of the offspring even in the absence of a readily identifiable need for intervention.

Conclusions

In summary, our initial human studies with TCDD were designed to explore the role of inflammation in the loss of progesterone sensitivity that had been identified as a key

component in the pathophysiology of endometriosis (reviewed by [14]). In turn, we developed our murine model to determine whether an early life toxicant exposure may lead to the development of the progesterone-resistant reproductive tract phenotype associated with this disease [50]. It is perhaps not surprising that the pregnancy studies we conducted in our toxicant exposure model suggested that the endometriosis-like phenotype not only affects fertility in female mice but also impacts their pregnancy outcomes [4]. Our murine model provided us with an unexpected experimental system to unravel the influence of reduced progesterone responsiveness on the various etiologies of PTB that have been identified in humans that directly or indirectly relate to inflammation. The superimposition of any number of well-recognized risk factors for PTB (eg, infection, socioeconomic position, and stress among others) upon a maladaptive inflammatory phenotype would be expected to trigger the cascade of the final common pathway leading to PTB. Clinical translation of the murine studies described herein would suggest that human therapy should shift fundamentally from abatement once the inflammatory cascade is set in motion (tocolytics, supplemental progesterone) to a preemptive interventional strategy involving both partners to restore homeostasis in the proinflammatory/anti-inflammatory balance before the onset of pregnancy.

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