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Epigenetics as a Driver of Developmental Origins of Health and Disease: Did We Forget the Fathers?

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What are the effects of our environment on human development and the next generation? Numerous studies have provided ample evidence that a healthy environment and lifestyle of the mother is important for her offspring. Biological mechanisms underlying these environmental influences have been proposed to involve alterations in the epigenome. Is there enough evidence to suggest a similar contribution from the part of the father? Animal models provide proof of a transgenerational epigenetic effect through the paternal germ line, but can this be translated to humans? To date, literature on fathers is scarce. Human studies do not always incorporate appropriate tools to evaluate paternal influences or epigenetic effects. In reviewing the literature, I stress the need to explore and recognize paternal contributions to offspring's health within the Developmental Origins of Health and Disease hypothesis, and coin this new concept the *Paternal Origins of Health and Disease* paradigm (POHaD). A better understanding of preconceptional origins of disease through the totality of paternal exposures, or the *paternal exposome*, will provide evidence-based public health recommendations for future fathers.

1. Introduction

More than 3 years ago, in a Think Again paper in BioEssays, a number of developmental windows were suggested in which the epigenome may play an important role in translating environmental messages on human and animal health.[1] The in utero environment is one particular window that has been studied widely in the field of Developmental Origins of Health and Disease (DOHaD). Early-life exposures through maternal diet, lifestyle, and other environmental conditions have indeed proved to be important for growth and health of offspring. However, what is often overlooked is the potential effect of pre- or periconceptional parental lifestyle and other exposures. Periconceptional exposure to food deprivation in the Dutch famine cohort or seasonal dietary circumstances in the Gambian cohort showed strong associations with altered DNA methylation in offspring.[2,3] In the case of the Dutch hunger study, early undernutrition has been related to poor health outcomes, including obesity,[4] hypertension,[5] cardiovascular diseases,[6] and cancer[7] in the offspring. Similarly, human parental exposure to psychological trauma has been considered as a risk factor for offspring's wellbeing.[8] However, most human studies have not addressed the potential importance of preconceptional or paternal exposures, although it is likely that future fathers experienced the same harmful nutritional or stressful condition before conception as mothers did. Animal data provide evidence for an environmentally induced epigenetic alteration in gametes (sperm or oocyte), which may influence embryogenesis, fecundity or health in the next generation(s). In some cases, epimutations have been uncovered in sperm cells and in offspring tissues after paternal exposures to certain dietary conditions, stressful conditions, environmental contaminants, etc… The literature contains numerous papers on animal data related to environmental influences, and reviews or other reports have summarized and discussed promising theories on epigenetic mechanisms explaining the link between parental exposures and germline or offspring outcomes.[9–19]

Unfortunately, in humans this area has remained underexplored. The lack of research interest in the role of the father is often strikingly apparent in that most conferences related to early exposures do not have a section on paternal influences. I here focus on the few epidemiological studies that included (future) fathers and verified epigenetic outcomes to understand the mechanism of potential transgenerational inheritance of early exposures in human beings. The approaches needed in epidemiological settings are different compared with simplified animal models that have been used in the field of transgenerational epigenetics. While my main goal was to explore an epigenetic link between exposure in the father and child health, I also included some studies in which an epigenetic mechanism was not confirmed but could be suggested as being involved. Some results on animal studies have been included to fill the gaps. Further, I added critical viewpoints to highlight difficulties in current study designs where paternal influences have been

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investigated in humans. Often, the original study was not designed to explore paternal lifestyle or exposures. Moreover, if attempts to study influences from paternal environmental exposures were made, some drawbacks in their designs may lead to data misinterpretation. To summarize the totality of exposures a father may experience preconceptionally, I suggest the term *paternal exposome*, as the origin of health and disease from paternal influences (or POHaD).

2. Does the Environment Influence Male Fertility and Sperm Characteristics?

2.1. Male Infertility

Globally, infertility affects about 10% of couples; men are responsible for about 50% of these reproductive issues.[20] A variety of disorders are associated with male infertility. Also environmental factors have a negative impact; ranging from obesity, diet, lack of exercise[21] to indoor[22] or outdoor pollution.[23] A temporal decrease in semen quality has been reported in many countries.[24] It is still unclear if, how, and to which extent each specific environmental condition could be related to this global decline. Moreover, infertility rates may also be a consequence of a multitude of several exposures together: the *exposome*. Most reports only show clinical outcomes, whereas the potential role of an intermediate factor, such as the epigenetic system, has rarely been assessed.

2.2. Is the Human Sperm Epigenome Sensitive to Lifestyle-Related Factors?

2.2.1. Obesity-Related Factors

In a cross-sectional study of 67 young and healthy volunteers an association was shown between overweight/obesity and DNA methylation patterns in sperm.[25] Obesity is a multifactorial condition. Comprehensive studies are needed to explore which obesity-related factor could lie at the origin be of this preliminary finding. This and other human-based studies are summarized in **Table 1**. The research group led by Barres provided a comprehensive epigenetic approach on sperm samples from obese and lean Danish men. They found significant differences between these two subgroups.[26] However, the number of subjects were small (a maximum of 10 obese and 13 lean men) and not all analyses were performed in the complete study population. Furthermore, exclusion criteria were slightly different in lean versus obese men, which may have caused bias. In a smaller group of six bariatric patients, Barres's group showed that surgery-induced weight-loss interventions were associated with epigenetic changes in sperm cells. However, it cannot be concluded that these reported epigenetic changes in sperm were due to weight loss only. Other factors could be related to this intervention as well, such as metabolic changes over time, stress, dietary, or lifestyle changes, decreased uptake of vitamins or intake of specific supplements, etc. Setting up an intervention study of this kind is quite complex. Systemic responses to surgery may vary individually. Nevertheless, studies like these are an indispensable step toward understanding the dynamic mechanisms of the sperm epigenome and more specifically its transcriptome. Barres' study should be repeated in other and larger populations, where potential confounders are traced. An alternative strategy could be to include a control group of patients who underwent surgery for other reasons than obesity, if possible, with comparable impact on the intestinal tract or metabolic system.

2.2.2. Exercising Programs in Men

Another intervention study in Australia by Denham et al. assessed global and genome-wide DNA methylation in sperm

Table 1. Environmental conditions and sperm epigenome. Few studies have explored epigenetic effects in human sperm from environmental conditions.

Reference	Study design	Subjects	Geographic area	Exposure	Epigenetic outcome
Soubry et al., 2017[46]	Cross-sectional	67 volunteers	NC, USA	Flame retardants (OP)	DNA methylation at 12 DMRs
Shnorhavorian et al., 2017[58]	Retrospective	9 patients (exposed) versus 9 non-exposed	5 States, USA versus 1 State, USA	Chemotherapy	DNA methylation at DMRs (MeDIP-Seq analysis)
Soubry et al., 2016[25]	Cross-sectional	67 volunteers	NC, USA	Overweight/obesity (BMI)	DNA methylation at 12 DMRs
Donkin et al., 2015[26]	Cross-sectional; Intervention	23 volunteers; 6 bariatric-interventions	Denmark	Obesity; bariatric intervention	Genome-wide DNA methylation, RNA expression, Histone positioning
Denham et al., 2015[27]	Intervention	13 interventions versus 11 controls	Victoria, Australia	Exercise (3 months)	Global DNA methylation, Genome-wide DNA methylation
Marczylo et al., 2012[57]	Cross-sectional	10 volunteers from Fertility clinic	UK	Smoking	miRNAs
Tunc et al., 2009[29]	Intervention	45 infertile men	South Australia	Supplements of folate and antioxidants	Global DNA methylation
Ouko et al., 2009[52]	Cross-sectional	16 volunteers	Johannesburg, South-Africa	Alcohol (self-reported)	DNA methylation at 2 DMRs

using an ELISA assay and the Illumina 450 K BeadChip. Denham et al. showed that exercise training of three months in 13 healthy volunteers resulted in a decrease in global sperm DNA methylation of nearly 7%. Genome-wide DNA methylation analyses showed changes at CpG sites of over 4000 genes.[27] Noteworthy, the Illumina 450 K platform is widely used for measuring genome-scale DNA methylation in humans. But, because of its human-specific application a comparative analysis between these findings and murine models is hindered. Denham et al. reported that increased methylation was observed at CpG sites of genes related to schizophrenia, Parkinson's disease, and some cancers such as cervical cancer and leukemia. Although the data are promising, they miss some transparency and thoroughness. Base-line data of controls and intervention-cases were significantly different (about 15%). Although the authors report no significant change in global DNA methylation in the control group after 3 months, a slight increase can be noted and no *p*-value was shown to comply with their conclusion. Further, controls were asked not to change their dietary or life-style patterns, but this was not verified at the end of the study. Additionally, no quantified genome-wide DNA methylation analyses were performed in these 11 men who did not follow an extensive exercising program. In brief, it cannot be excluded that other environmental factors, including seasonal dietary differences, or nutritional changes due to unknown reasons could have influenced their results. The authors further report that a number of imprinted genes were differentially methylated after the exercise program. For instance, DNA methylation % at the *IGF2* DMR was slightly decreased after intervention. They suggest that abnormalities at this particular region could be linked with metabolic or neurological diseases, and speculate that exercise training may remodel the sperm epigenome and reduce the risk for these diseases in the offspring. However, care should be taken before drawing this conclusion. Especially, given that a decrease in DNA methylation percentage at the *IGF2* DMR in sperm, as was measured after 3 months of training, is expected to be detrimental rather than beneficial. If correctly reprogrammed or remethylated during spermatogenesis, mature sperm should be 100% methylated. This contradicts the suggestion in the Denham et al. study.

A similar study by Ronn et al. in 23 healthy Swedish men showed that a 6-month exercise intervention program caused differential DNA methylation at more than 7000 genes and altered mRNA expression in adipose tissue.[28] Although tissues of interest vary in different studies, it would still be valuable to compare outcomes of these studies. Interestingly, Ronn et al. extended their study by an additional in vitro experiment on adipocytes. Genes that were found to have increased in DNA methylation profiles after the exercise intervention program were experimentally silenced in vitro. This functional test showed that lipogenesis was increased, suggesting that adipocyte metabolism was triggered by the exercise program.[28] The addition of a functional in vitro test provided valuable information and is a fine example of integrative research. Hence, where epidemiological study designs fail to address all aspects of the research question, in vitro experiments may well provide complementary information.

2.2.3. Paternal Diet

As was suggested above, nutritional changes may coincide with exercise interventions. However, studies in men measuring epigenetic effects after dietary interventions are even rarer. A study in 45 infertile men indicated that a 3-month supplementation with folate and antioxidants significantly improved sperm quality and increased global sperm DNA methylation in such a way that methylation was restored.[29] No analyses were performed on control subjects at the end of these 3 months. Although study populations and intervention types differed in the Denham et al. and Tunc et al. studies, it remains unclear why in the first study a decrease in global sperm DNA methylation was correlated with an improvement of sperm epigenetics,[27] while in the second study a global increase in DNA methylation was described as being beneficial.[29] At least, control samples should be included in all analyses, to prevent effects from confounding factors. The field urgently needs larger, standardized, and more comprehensive studies. To our knowledge, no other intervention studies have been published in which epigenetic characteristics of sperm in young or fertile men were explored after well-documented dietary changes. Hakonsen et al. showed that a combined diet and exercise weight-loss program during three and a half months in 43 obese men improved sperm morphology and total sperm count, if subjects succeeded in losing a sufficient amount of weight.[30] However, no epigenetic data were collected.

Animal data provide evidence for involvement of the male germ line after dietary changes in fathers. Ng et al. in 2010 found that paternal consumption of high-fat diet (HFD) induces glucose intolerance and DNA methylation changes of a key pancreatic islet gene, Il13ra2, in offspring rat.[31] In 2014, they showed in the same model that the transcriptome of retroperitoneal white adipose tissue was also concomitantly affected in offspring.[32] Unfortunately, they did not provide sperm analyses to explore potential involvement of components of the epigenetic system in the transmission of this dietary-related exposure. Palmer et al. reported in a mouse model that HFD in males causes increased levels of acetylated H3K9 in sperm.[33] More recently, diet-induced paternal obesity has also been shown to alter sperm microRNA content in mice.[34] Protein restriction in mice affects sperm small RNA (sRNA) levels, such as transfer RNAs (tRNAs).[35] Interestingly, Grandjean et al. in 2015 demonstrated that microinjection of testis or sperm RNA of male mice fed with a Western-like diet into naive one-cell embryos caused establishment of a Western-like diet-induced metabolic phenotype in the offspring. They further showed that one specific small RNA, miR19b, could be at the origin of this food-induced transgenerational inheritance of acquired phenotypic traits.[36] Similarly, Chen et al. showed that injection of sperm tRNA-derived small RNAs (also referred as tsRNA) from mice that had been administered a HFD into normal zygotes caused altered gene expression of metabolic pathways in early embryos and generated metabolic disorders in offspring.[37] Because sperm is assumed to be transcriptionally inactive, it has long been believed that it lacks functional RNAs. Yet, the latest technologies have changed this idea. Sperm carries a large and varied RNA population. Further research on small

RNAs promises better insights in how sperm may respond to the environment and transmit these messages to the zygote.

2.2.4. Paternal Stress

The epigenetic system could also function as an adaptive way to manage stress from earlier or ancestral exposures. For instance, paternal stress in mice-induced hyperglycemia in offspring due to increased expression of phosphoenolpyruvate carboxykinase (PEPCK), a rate-limiting enzyme of neoglucogenesis in the liver. Hypermethylation at the promoter of the *Sfmbt2* gene in sperm and a decrease in miR-466b-3p expression of offspring liver provided the epigenetic explanation behind this observation.[38] Remarkably, Gapp et al. suggested through an experiment in mice that small non-coding RNAs (sncRNAs) are also able to transduce traumatic stress messages from father to offspring.[39] Animal data are not limited to these examples described above. As stated in the Introduction, several reviews have summarized and discussed the long and growing list of animal data to help understand how the environment can alter the germ line epigenome, and induce phenotypic changes or diseases in the offspring. However, to our knowledge, no human studies have explored a function of RNA fragments in the transmission of dietary or stress conditions from father to child, yet.

2.3. Is the Human Sperm Epigenome Sensitive to Environmental Pollutants, Alcohol, Smoking, and Chemotherapy?

An important group of relatively new environmental pollutants are organophosphate (OP) flame retardants. OPs are becoming more widely used in textiles, electronic devices, housing insulation materials, paints, etc. as a replacement for the known toxic brominated flame retardants (such as PBDEs). However, measurements of indoor dust samples and analyses of urinary metabolites in human subjects strongly suggest that OPs leak into the environment and are absorbed by the human body, potentially through hand-mouth contact, dust ingestion, and/or inhalation.[40–44] Urinary biomarkers for flame-retardants or organochlorine pollutants are associated with male reproductive health and sperm parameters.[45] In a recent study, we collected sperm and urine samples from 67 young healthy volunteers in North Carolina, USA. Urinary metabolites of OPs were measured using liquid-chromatography tandem mass-spectrometry, and sperm DNA methylation was quantified at multiple CpG sites of regulatory differentially methylated regions (DMRs) of several imprinted genes, using bisulfite pyrosequencing. A significant association was found between exposure to OPs and epigenetic abnormalities.[46] The cross-sectional design of this study was a limitation. Hence, no causal relationship could be warranted. Associations were small and provided no cause for direct concern at an individual level. However, at a population level – if results from animal models can be transferred to humans – these findings can be translated into increased incidence of chronic disorders in subsequent decades.[47–50] Interestingly, this study further showed that if an individual was exposed to more than one chemical compound, the association was stronger. This means that a multiplicity of environmental substances foreign to the human body may do more harm than what is often measured for a single compound in laboratory conditions. Some rare animal experiments on low-dose exposure of more than one compound support this hypothesis.[51]

Chronic alcohol use has also been associated with sperm epigenetic aberrancies, such as DNA methylation at the *IGF2* and *H19* DMRs.[52] Although this cross-sectional study only used self-reported alcohol consumption, and only 3 Caucasian and 13 African men volunteered for this study, animal studies have partly confirmed this effect.[53,54] More specifically, animal data indicate the complexity of epigenetic changes, not only involving methylation alterations, but also altered chromatin remodeling and different classes of non-coding RNAs (ncRNAs).[55,56]

In humans, some indication has been found that cigarette smoke induces differential miRNA expression in spermatozoa.[57] Unfortunately, this finding was based on 5 smokers and 5 non-smokers only, and the authors provided little information regarding potential confounders and dose of exposure (e.g., number of cigarettes per day). It would be informative to continue this research in a larger cohort.

Recently, sperm from young men treated with chemotherapy for osteosarcoma in their adolescence was compared with sperm from a control group.[58] Nine cancer survivors were selected from five collaborating hospitals in different States of the US, where they had been treated in the past. Sperm from a control group ($n = 9$) was compared with sperm from these chemotherapy recipients, more than 2 years after therapy. Genetic mutations (copy number variation) as well as DNA methylation differences were verified in both subgroups. Sperm DNA methylation patterns were altered in cancer survivors, compared to those in men who had never received chemotherapy. Unfortunately, control subjects were selected from only one State and were more than 10 years older than the men who had been treated with chemotherapy. Aging in men has been associated with DNA methylation changes.[59] Because a 10-year difference may cause minor but measurable differences, it is recommended to match the comparison group by age. DNA methylation is a potential age-biomarker or predictor; depending on the methods used the accuracy of age prediction is 5 years or less.[60] Further, to reduce cost and because the number of subjects was low, pooling of sperm samples was performed. Regardless of these limitations, results are promising. Still, it cannot be excluded that the condition itself (cancer at an early age) could be associated with a change in sperm epigenome. First, adolescence (time of chemotherapy) is not expected to be a major window of susceptibility wherein important methylation reprogramming of sperm occurs; hence, "stable" epigenetic marks are not expected to happen in this period of life.[9] Second, in the context of the DOHaD theory and the potential for epigenetic inheritance from preconceptional or in utero exposures, it cannot be excluded that some ancestral or early environmental exposure may have affected offspring health, including susceptibility to cancer, as well as epigenetic changes in other tissues, such as the testes. In other words, it can be speculated that a trans- or intergenerational effect from (grand) parent to son may have caused cancer in these men, as well as epimutations in sperm. Nevertheless, Skinner et al.'s results

provide the tools to generate new hypotheses on paternal influences from cancer treatment in humans; this urges epidemiologists to pursue this line of research.

3. Do Environmental Conditions of a Future Father Affect Offspring Health on the Long-Term?

3.1. A New Paradigm: Paternal Origins of Health and Disease (POHaD)

Paternal environmental conditions not only negatively affect sperm, they also have an influence on pregnancy success rates and offspring health. Some milestone longitudinal studies in humans provided evidence for transmission of effects from early exposures through the male germ line. The historical Överkalix study showed that longevity of grandchildren was determined by the grandparent's diet during pre-puberty, in a sex-specific way.[61] The ALSPAC study in the UK showed that early smoking in fathers was related to increased risk of obesity in their sons.[62] These data suggest the importance of early life exposures in men and involvement of epigenetic mechanisms. However, no epigenetic tests have been performed in these fathers or in their offspring. As suggested above, cigarette smoke may cause epigenetic damage in sperm cells and paternal cigarette smoking has been related to DNA damage in cord blood of the offspring.[63] However, the exact mechanism in germ-line transmission has not been uncovered yet. Continuing into this direction may help the field understanding the epigenetic mechanisms involved in transgenerational effects in humans.

Table 2 summarizes current literature on paternal exposures and offspring outcomes in humans, including a potential epigenetic link. Theoretically, sperm cells – or seminal fluid – are the only vectors determining how environmental influences can be transferred from father to child. The exact mechanisms of how "hidden messages" in sperm are transferred from father to child are not completely understood, but the field has now accepted that the epigenome is responsible for this intriguing process. It is hypothesized that plasticity is part and parcel of the epigenetic machinery, which allows the environment to leave a mark on the germ line that will or will not be passed on to the next generation. Whether this will happen – and especially how this process occurs – is the urgent question posed by researchers in the field. Paternal exposures and subsequent phenotypic and (epi)genetic changes in offspring are increasingly being considered as a new theory or an extension of the DOHaD concepts.[64] Given that these DOHaD concepts are being used to

Table 2. Fathers and offspring health. In humans, few studies search for an epigenetic link between paternal exposures and offspring health. Here, literature is provided where epigenetic correlations have been shown or where one could hypothesize there is an epigenetic link.

Reference	Study design[a]	Subjects	Geographic area	Paternal exposure	Offspring outcome	Epigenetic analyses
Mejia-Lancheros et al., 2017 (poster)[67]	Cohort	213 children of age 5, 148 children of age 9	Ireland	Vitamin D	Weight and height	None
Svanes, et al., 2017[78]	Population-based cohort	24 168 offspring	Northern-Europe	Smoking	Asthma	None
Wu et al., 2017[81]	Cross-sectional	50 IVF couples	MA, USA.	Phthalates	Embryo quality	None
Yehuda et al., 2016[73]	Retrospective cohort	31 Holocaust offspring, 9 Jewish US citizens (demographically matched)	USA	Holocaust (stress)	–	DNA methylation at *FKBP5* gene
Feinberg et al., 2015[93]	Cohort	44 fathers with at least one autistic child	4 sites, USA	Unknown source; sperm was analyzed	Autism in early life of a subsequent child	DNA methylation in sperm
Northstone et al., 2014[62]	Birth cohort	9886 fathers	Avon, UK	Smoking	Obesity in adolescents	None
Soubry et al., 2013[65]	Birth cohort	79 fathers	NC, USA	Obesity/ overweight	Unknown; cord blood was analyzed	DNA methylation at 2 DMRs
Soubry et al., 2013[66]	Birth cohort	79 fathers	NC, USA	Obesity/ overweight	Unknown; cord blood was analyzed	DNA methylation at 7 DMRs
Hultman et al., 2011[92]	Population-based cohort	1 035 487 offspring	Sweden	Age	Infant/childhood autism	None
Kobayashi et al., 2009[82]	Case-only	17 ART aborted embryos with imprinting errors	Japan	Unknown source; sperm was analyzed	Aborted ART embryos with imprinting errors	DNA methylation at 7 DMRs and repetitive sequences (LINE1 and Alu)

[a]In some reports analyses were performed on a wider study cohort or design; however, a focus was set on the specific or "nested" design relevant to the research question. IVF, in vitro fertilization; ART, assisted reproductive technology.

guide policies supporting parents and children's health, I believe it is of public interest to also explore the paternal side of DOHaD-related research, or more specifically, to introduce a new field which we here coin Paternal Origins of Health and Disease (POHaD).

3.2. Can Paternal Obesity or Nutrition Influence Offspring Health?

Earlier findings in humans showed a correlation between paternal obesity or related lifestyle factors and epigenetic abnormalities at the level of regulatory regions of imprinted genes in newborns.[65,66] Unfortunately, no similar studies have been performed on other birth cohort data. Most recently, an Irish study by Mejia-Lancheros et al. in 148 fathers showed that preconceptional paternal vitamin D intake was positively associated with offspring's height and weight at the age of 5 years, independent of maternal vitamin D intake and other potential confounders.[67] The latter finding is unique in humans, given that most research on potential mechanisms of vitamin deficiency or supplementation and offspring health originates from animal models. For instance, if pregnant mice were exposed to a folate-deficient diet, offspring's sperm was more likely to have altered DNA and histone methylation. Remarkably, this diet did not alter morphology of adult testes or sperm count, but fertility was reduced and if offspring was produced by these males, birth and placenta defects were more common, compared to births after a control diet.[68] Other dietary interventions, such as a high-fat diet in rodents, may not only impair sperm quality, but delay cell cycle progression during preimplantation, reduce implantation rate, and/or produce smaller offspring.[69] Interestingly, improving paternal metabolic health through diet and/or exercise before conception restores these effects.[70,71] It seems that – in mice – exercising and/or dietary interventions, even for a short-term of 8 weeks (or two rounds of spermatogeneses), is sufficient to measure these improvements. For some unexplained reasons exercise-only interventions showed the highest improvement. Unfortunately, we still lack human studies to verify these findings. There have been some attempts to measure paternal diet in human birth cohorts. However, study designs and/or analytical approaches have not been published in peer-reviewed journals or were flawed. For instance, measuring paternal nutritional intake during the partner's pregnancy may not represent a father's regular dietary intake before conception. Furthermore, paternal and maternal food patterns or lifestyle are usually closely correlated, making it difficult to distinguish the origin of one particular food pattern or lifestyle aspect. Hence, careful attention should be paid to potential confounding by maternal diet. Therefore, if the study was not designed as a male-mediated one, findings need to be carefully interpreted if no adjustment was made for maternal diet.

3.3. Can Paternal Stress Affect Offspring Health?

Exposure to food insecurity and stress is another example of combined exposure that is difficult to untangle. For instance, during war or nature's disasters humans experience a multitude of important changes from their environment. Survivors of the Holocaust who experienced persistent undernutrition and psychological trauma in World War II have been studied in Israel and in the US. Fridman et al. examined 32 female Holocaust survivors and 47 daughters in Israel; about an equal number of families were included without this traumatic experience in the past.[72] After studying mental health, physical health, and cognitive functioning no differences were found between offspring from exposed and non-exposed mothers. No epigenetic tests were performed in these subjects and fathers or sons were not included in this study. Yehuda et al. in US citizens studied 32 Holocaust survivors (20 mothers and 12 fathers) and 31 offspring (16 daughters and 6 sons).[73] A relatively small group of US-Jewish subjects were used as controls, including 6 mothers, 2 fathers, 8 daughters and 1 son. DNA extractions from whole blood showed differential methylation at *FKBP5*, a stress-related gene, in offspring from exposed parents versus controls. Exposed men and women were mostly husband and wife. Hence, both were exposed and a sex-specific epigenetic effect could not be verified from these data. Besides stress, other unmeasured potential confounders could be at the origin of this differential *FKBP5* methylation. For instance, it is not clear how conditions were in the control group during the war. Which other factors differed between these two subgroups? Did they move to the US in the same period as cases did? Was their nutritional status better? Nevertheless, Yehuda et al.'s point can be strengthened by their earlier findings on another stress-related exposure. Individuals who suffered posttraumatic stress disorder after the World Trade Center attack in 2001 had increased expression of FKBP5.[74] Unfortunately, paternal and offspring data are not available yet. Future studies of severe trauma on both parents and offspring may help uncover and understand epigenetic influences.

The ice storm of 1998 in Quebec also provided data to explore potential epigenetic effects from parental stress in humans from natural disasters. Cao-Lei et al. studied offspring from mothers who were pregnant during the ice storm or who conceived within 3 months after onset of the storm.[75] Using genome-wide DNA methylation analyses they found a dose-response effect between the degree of Prenatal Maternal Stress (PNMS) and DNA methylation levels in different cell types in children of an average age of 13. Recently, Cao-Lei et al. suggested a protective role of the epigenome in response to PNMS. Maternal stress was not only associated with DNA methylation changes but also with lower BMI and adiposity in offspring.[76] Unfortunately, potential effects through paternal stress have not been explored in this cohort as well. Furthermore, it would have been informative to report the results of a subgroup of children who were conceived after the storm. Notably, although the Quebec ice storm caused tremendous stress, it cannot be excluded that limited access to food was another issue these people had to cope with. Therefore, a nutritional "stress" factor should be considered as well. Until now, it has remained unclear how these (epigenetic) messages are transferred; through the mother, the father, or both. Regardless of the difficulties involved in untangling maternal and paternal, stress, dietary or lifestyle influences it is

certainly worthwhile to proceed with more research in this direction.

3.4. Can Offspring Be Affected by Paternal Smoking, Alcohol Consumption, and Other Environmental Exposures in Humans?

Data from the "Avon Longitudinal Study of Parents and Children" (ALSPAC) show that adolescent sons of fathers who started smoking before puberty are at high risk of being obese.[62] Although no underlying biological mechanism has been shown yet, this fascinating finding suggests that cigarette smoke metabolites may induce epigenetic changes during prepubertal production of spermatogonia in testes of fathers.[77] Interestingly, this hypothesis is corroborated by a most recent finding by Svanes et al., who show that the earlier the father started smoking, the higher the risk for non-allergic asthma in the offspring.[78]

As indicated above, alcohol consumption affects sperm. In mice offspring, hearing loss has been related to alcohol intake.[79] Most recently, Rompala et al. showed that paternal alcohol administration in mice prior to conception affected inheritances of complex behaviors in offspring in a sex-specific way. Male offspring showed a decreased preference for ethanol drinking, an increased sensitivity to the anxiolytic effects of ethanol, and increased expression of the brain-derived neurotrophic factor (BDNF) gene in brain. The authors suggested involvement of germ-line epigenetic mechanisms, but this has not been verified yet.[80]

The drive to further explore this intriguing connection between exposures in men and germ-line or offspring outcomes in humans appears to be largely absent at the moment. In general, there are no public concerns regarding the effect of smoking or drinking behavior in men on their future offspring, but studies described above together with experimental designs in animals urge the field to take action, to include paternal lifestyle factors in our birth cohorts, and to inform public health officials where needed. Current precautions advised for future mothers before conception may be insufficient and recommendations should be broadened to both parents.

A recent study on American couples undergoing IVF treatment showed an association between paternal exposure to phthalates and poor quality of blastocysts.[81] Here too, a potentially underlying epigenetic mechanism was not further verified. In general, few epidemiological studies have explored epigenetic effects from environmental toxins or pollution on sperm epigenetic mechanisms or offspring health. Notably, a comparative analysis in Japan on couples who had lost a child through abortion showed that methylation abnormalities in embryos of about 7 weeks old could be traced back to the same methylation aberrancies in paternal sperm.[82] Unfortunately, this study missed comparative analyses on fathers from aborted embryos without imprinting errors. Although purely hypothetically, transferred methylation defects from sperm to embryo could be due to environmental factors from the father.

Other public health concerns are preconceptional occupational exposures in men. Literature provides ample evidence for a link between paternal occupations and offspring health. Children from painters,[83] electronic workers,[84,85] and soldiers exposed to chemical weapons[86] have increased risk of congenital abnormalities. A case-control study on congenital malformations, reported an odds ratio of 5.6 (95%CI: 2.8–11.4) among children born to fathers exposed to solvents in the workplace and an odds ratio of 3.4 (95%CI: 1.97–5.92) if fathers had been exposed to pesticides preconceptionally.[87] Paternal occupational conditions have also been related to cancer in young children.[88,89] For instance, a case-control study of 65 chemical substances and their potential to cause neuroblastoma reported positive associations if the father had been exposed to turpentine (OR: 10.4, 95% CI: 2.4–44.8) or paint thinner (OR: 3.5, 95%CI: 1.6–7.8) during a 2-year period before birth.[90] Occupational exposures of a different kind, such as ionizing radiation, have also been linked with childhood cancer. Children born to fathers who had worked at a nuclear site in the UK before conception showed higher rates of leukemia and non-Hodgkin's lymphoma compared to the prevalence of these disorders in national health records. Demographic conditions or intermediate genetic mutations have been proposed as potential reasons for these relationships, but often sufficient evidence is lacking. In the case of workers at the nuclear facility in Sellafield (UK) – a still unsolved mystery of increased cancer incidence in their children – germline minisatellite mutations rates were investigated and results failed to provide a reason for the increased cancer incidence.[91] So far, no epigenetic tests have been performed in fathers who worked at this nuclear plant, or in similar conditions.

In general, literature provides ample evidence for a link between paternal occupation and offspring health, but there have been no clear explanations for these observations. In Soubry et al. it was suggested that inherited epigenetic conditions could at least contribute to this.[9] However, till now, not a single human study has investigated this hypothesis in this particular population. I therefore did not include occupational exposures in Table 2.

3.5. Which Other Paternal Factors May Be Related to Offspring Health?

Some unexplained disorders have been associated with paternal age, artificial fertilization procedures, or other earlier exposures. For instance, advanced paternal age has been associated with infant and childhood autism.[92] Autism Spectrum Disorder (ASD), in particular, has been proposed as an "environmental" disease where epigenetics may play an important role.[93] Interestingly, Feinberg et al. showed that in families of which at least one child had already been diagnosed with ASD, paternal sperm was differentially methylated at several DMRs if infants had a strong indication to develop ASD in their first year of life, compared to fathers of children who were found to be negative for these specific assessments. A limitation that could be attributed to this study is its temporal design. Although it is not always feasible to do otherwise, sperm samples were collected several months after conception. By exploring these autism-enriched families, the authors demonstrated an alternative way to study relatively rare disorders such as ASD.[93] However,

results cannot be generalized to a wider population – as is the case in the usual large birth cohorts. Still, Feinberg et al.'s approach does offer a new venue to be further explored in larger samples. Given it is currently unknown which environmental factor provokes development of autism, or other diseases, this approach could also be applied in exposure-oriented studies. Subjects could be selected by geographic area where the relative risk to a particular exposure is higher, where people experience specific work-conditions, or undergo a specific treatment. As long as the limitations inherent in these "selected-cohorts" are taken into account, this method may help identify important epigenetic players.

4. Conclusions and Outlook

Scientists in the field have become aware that environmental exposure of future fathers can adversely affect pregnancy outcomes and their offspring. The processes involved in transferring an environmental message through male germ cells is not well understood and still underexplored, especially in humans. There is strong evidence from animal models for involvement of paternal factors in the origin of health or disease in offspring through intermediate epigenetic mechanisms. The increasing number of reviews is proof of the expansion of animal data on this topic. Mechanisms have been proposed on how exposures can change the epigenome of the germ line and be transmitted to the next generation(s). Future animal models could be used to modify sperm epigenetically in a specific and controlled way to generate offspring, and to observe the outcomes. A guideline for experimental designs in animals has been published recently.[94] While results from animal data are promising, caution should be taken in extrapolating findings from animal models to humans. As noted earlier,[64] a reason for concern is the fact that animal studies often provide the rationale for clinical studies or treatment opportunities. And as stated above, the issues one may encounter in human studies are different and more complex than what can be achieved through animal models. Well-crafted human study designs are indispensable to verify findings from animal data.

The first remarkable finding from reviewing literature is that most studies on human subjects still explore environmental conditions and potential corresponding epigenetic intermediate factors in mother-child dyads only. Paternal influences are rarely included in longitudinal studies, such as birth cohorts. These studies may thus miss some important determinants that could explain subfertility, failed pregnancy success (such as miscarriage or premature labor) or offspring health conditions. Secondly, in the rare studies that did include paternal factors, study designs or analyses were not always optimal. Including paternal influences imposes extra challenges. Difficulties include the fact that maternal and paternal exposures are often closely related to each other, making it difficult to distinguish whether the effect of one specific exposure factor is due to maternal or paternal influence. Hence, analyses of studies not designed to explore paternal influences at the original set-up may result in misinterpretation of the data. Including outcomes such as known players of the epigenetic machinery may urge the field to make these explorative or observational studies as comprehensive as possible. Sharing data from expensive high-throughput sequencing analyses of small cohorts or from different tissues or cell types is warranted. In order to reach this, efforts are needed from research funding agencies to support creation and operation of platforms or focus groups in this young but growing field.

It is expected that more data on paternal influences will follow in the next few years, but we need to remain careful in interpreting them. New areas or "hot topics" are often prone to overestimate results. Hence, a critical view remains indispensable and researchers should not hesitate to report negative results as well. This will prevent a potentially biased impression of reality. Ultimately, future research on paternal exposures will increase knowledge on this new but intriguing POHaD paradigm.

Abbreviations

ART, assisted reproductive technology; ASD, autism spectrum disorder; DOHaD, developmental origins of health and disease; DMR, differentially methylated region; *IGF2*, insulin-like growth factor 2; IVF, in vitro fertilization; OP, organophosphate; OR, odds ratio; POHaD, paternal origins of health and disease.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

DOHaD, epigenetics, paternal exposome, POHaD

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