Paternal periconception metabolic health and offspring programming

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Abstract

The association between maternal metabolic status at the time of conception and subsequent embryogenesis and offspring development has been studied in detail. However, less attention has been given to the significance of paternal nutrition and metabolism in directing offspring health. Despite this disparity, emerging evidence has begun to highlight an important connection between paternal metabolic well-being, semen quality, embryonic development and ultimately adult offspring health. This has established a new component within the Developmental Origins of Health and Disease (DOHaD) hypothesis. Building on the decades of understanding and insight derived from the numerous models of maternal programming, attention is now becoming focused on defining the mechanisms underlying the links between paternal well-being, post-fertilisation development and offspring health. Understanding how the health and fitness of the father impact on semen quality is of fundamental importance for providing better information to intending fathers. Furthermore, assisted reproductive practices such as *in vitro* fertilisation rely on our ability to select the best quality sperm from a diverse and heterogeneous population. With considerable advances in sequencing capabilities, our understanding of the molecular and epigenetic composition of the sperm and seminal plasma, and their association with male metabolic health, has developed dramatically over recent years. This review will summarise our current understanding of how a father's metabolic status at the time of conception can affect sperm quality, post-fertilisation embryonic and fetal development and offspring health.

Introduction

Over recent decades, human and animal model studies have highlighted the significance of the *in utero* period in shaping patterns of fetal development and offspring long-term health (1). Investigations into maternal exposure to different environmental factors during pregnancy have shown that the offspring can display an increased propensity for developing a range of non-communicable conditions such as cardiovascular disease (2), insulin resistance and obesity (3) and certain behavioural disorders (4). The Developmental Origins of Health and Disease (DOHaD) field has expanded to investigate a range of environmental and lifestyle challenges, as well as defining the sensitivity of specific 'windows' before, during and even after pregnancy (5). One such window that appears to display specific sensitivity is the time around conception and early embryonic development. This preconception period, as defined in Fleming et al., (6), typically represents a time encompassing parental gamete maturation, fertilisation of the oocyte and development of the preimplantation embryo. The importance of the periconception period is highlighted by the fact that it encompasses a transition in developmental regulation, driven initially by the quality of the parental gametes before being directed by the embryonic genome. Underlying these fundamental developmental processes are dramatic reorganisations of the epigenetic status of the parental genomes, allowing a new embryonic pattern to be established which then determines subsequent fetal and postnatal development (7). Due to the dramatic epigenetic remodelling that takes place within the preimplantation embryo, understanding the consequences of programmed changes in offspring epigenetic status (DNA/RNA methylation, histone modifications, non-coding RNA populations) as a result of periconception environmental insults has become a significant focus in the DOHaD field (8).

The fact that the periconception period represents a time of both critical developmental importance for future offspring well-being, as well as heightened sensitivity to environmental perturbations, has significant implications for our current lifestyle and fertility. The global population is burdened by an increase in amount of people experiencing either over- or under-nutrition (9). In addition, an increase in the number of people actively delaying parenthood (10) has resulted in a general decline in fertility, highlighting the interplay between our modern lifestyle and its influence on our gametes and general reproductive health (11). Infertility now affects around 15% of couples in their reproductive age and its global rate has increased significantly in the period between 1990 and 2017 (12). Furthermore, the demand for infertility treatment using Assisted Reproductive Technologies (ARTs) such as *In-vitro* Fertilisation

(IVF) or Intracytoplasmic Sperm Injection (ICSI) has increased also (13). While the relationship between maternal diet, gamete quality and fertility has been studied in detail, the significance of male nutritional status and post-fertilisation embryo development has received less attention. Paternal obesity has been shown to negatively impact male endocrine function, sperm quality and genomic/epigenetic integrity, fertilisation capacity, embryonic development and offspring health (14). Similarly, studies have also shown that paternal undernutrition affects sperm quality and post-fertilisation development and offspring well-being (15). Therefore, a greater understanding of the paternal contribution to offspring development is needed if new parental strategies are to be developed to combat the rise in rates of global non-communicable disease.

In line with our increased interest in the role nutrition plays in the regulation of our reproductive fitness, our understanding of the interplay between our microbiome and the function of multiple physiological systems has grown also (16). Our microbiota, the populations of micro-organisms that live within and on our bodies, and the role they play in regulating multiple aspects of our health and well-being are of increasing interest. Within the gut, the microbiota regulate numerous aspects of metabolism; secreting hormones and metabolites which regulate processes such as appetite, glucose tolerance, insulin sensitivity and fat storage (17), all of which are connected to reproductive health. During pregnancy, the maternal microbiota shows significant changes in composition (18) and the female reproductive microbiota has been associated with a variety of gynaecological cancers (19). In addition, the maternal-offspring microbiome exchange at birth is critical in establishing the neonate's microbiome in postnatal life (20). Interestingly, in males, the seminal plasma has been shown to have its own microbiota, which is modifiable by diet (21). While the significance of the seminal plasma microbiota has yet to be defined, the role of the seminal plasma in modulating the maternal reproductive tract during preimplantation embryo development is becoming evident (22). As our metabolic health, microbiota and our reproductive fitness appear directly interconnected, the role of the microbiome in regulating fertility is one we will explore within this review.

Here, we review the growing body of data surrounding paternal nutritional status and the association with sperm quality, preimplantation embryo development and adult offspring health. Throughout this review, we aim to present and highlight evidence reported from both animal models and human studies discussing, where possible, the potential relationship(s) between them. Similar to studies exploring maternal programming, animal model studies have

been fundamental in understanding the mechanistic relationships between paternal health, reproductive fitness and offspring development. This has allowed for in-depth analysis of male gametogenesis, epigenetic status and regulation, in addition to customised experimental models designed to simulate real-world nutritional profiles and lifestyle conditions. These models have aided the characterisation of the mechanisms underlying the paternal programming of offspring health.

Paternal nutrition and reproductive fitness

Obesity, defined in humans as a BMI>30kg/m^2, is associated with adverse metabolic effects and higher cardiovascular disease risk (23). Obesity has also been linked to reproductive dysfunction and an association with male infertility (24). However, while conflicting effects of obesity on semen quality and fertility have been reported in men, multiple studies in animal (mostly rodent) models have shown negative effects of obesity of a high fat diet (HFD) on male reproductive fitness. Hammoud et al. demonstrated that with increasing BMI in men, the incidence of oligozoospermia increased also, with obese men having a 15.62% incidence compared to 5.32% for normal weight men (25). Disturbances in endocrine homeostasis underpinning spermatogenesis indicate one mechanism by which obesity-mediated alterations in hormonal profiles may alter male fertility. For example, obese males have been found to have decreased testosterone, inhibin B and increased oestrogen levels, all associated with impaired spermatogenesis (26). Furthermore, alterations in the balance of gonadotropin releasing hormone and luteinising hormone/follicle stimulating hormone (GnRH-LH/FSH) may further contribute to an obesity-related impairment of spermatogenesis by disrupting Leydig and Sertoli cell function (27). Additionally, fat accumulation in male obesity can increase scrotal temperature which impairs spermatogenesis, contributing to decreased sperm quality (28). Male obesity has also been associated with increased risk of sperm DNA damage (29). In mice, increased reactive oxygen species (ROS) production and higher levels of DNA damage in sperm of HFD fed males have been reported (30). Elevated levels of testicular ROS, and the subsequent increase in oxidative stress, have been shown to have detrimental effects on sperm integrity through the impairment of the sperm plasma membrane (31). Furthermore, the elevation of ROS and the resultant increase in sperm cytotoxicity and DNA fragmentation (32), have been shown to reduce sperm vitality and motility (33), lower levels of sperm capacitation (34) and diminish sperm-oocyte binding capacity (35).

The impact of paternal nutrition has on a male's reproductive health extends beyond pre-conception period and into the peri-conception period. An association between elevated paternal BMI and impaired embryo development has been identified in men and animal models. In men undergoing ART interventions, an increasing BMI was significantly associated with a decreased rate of embryo blastulation on day 5 of culture (36). Furthermore, the same study reported rates of pregnancy, embryo implantation and live birth decreased from 41.3% for men of a BMI <25 kg/m^2 to 22.6% for obese men (36). In rats, similar observations were reported for embryos derived from obese males, with HFD-induced obesity reducing the cleavage rates of preimplantation embryos. Furthermore, these embryos demonstrated an impaired ability to achieve developmental milestones *in vitro* and ultimately failed to achieve blastocyst expansion at an appropriate time-point (37). Early embryo cleavage dynamics have been associated with rates of on-going development and live birth within clinical ART settings (38). Recently, the association between being overweight in men and a reduction in fertility has been supported further through a large-scale meta-analysis of 115,158 study participants, revealing obese men had an increased likelihood of infertility (39). Similar to the metabolic insult of high fat and obesogenic diets, paternal undernutrition and nutrient-deficient diets have also demonstrated an impact on sperm quality and early embryo development. In mice, pre-implantation embryos from low-protein fed stud males were found to have a reduction in genes associated with metabolic homeostasis, particularly a decreased expression of genes involved in the AMPK pathway (40). Separately, paternal global dietary restriction found undernutrition in male mice resulted in a faster cleavage time in preimplantation embryos, yet reduced rates of blastocyst expansion were observed (41).

Paternal nutrition and offspring health

The influence of paternal nutritional status extends beyond alterations in early embryonic development and a number of studies in animal models have highlighted the deleterious effects paternal over- and under-nutrition have on the health of a male's offspring. In mice, perturbed patterns of fetal growth and skeletal formation have been reported in response to both paternal low protein and low folate diets (40, 42). Interestingly, in both studies, altered placental development was highlighted as one central regulator of the changes in fetal growth, mirroring observations from many maternal programming studies (43). Changes in expression of several hepatic genes for lipid metabolism have also been observed in response to paternal low protein diet (44). These differential growth and metabolic profiles seen during fetal development are then mirrored in postnatal life. In mice, a paternal 70% caloric restriction model, designed to

reflect the nutritional availability in of developing countries, increased levels of adiposity in male offspring adiposity as well as inducing dyslipidaemia (41). Similarly, a paternal reduction in caloric intake (reduced by 25%) in the rat resulted in an increase in displays of anxiety-like behaviours in the adult offspring in addition to inducing a reduction in food intake, weight gain and serum leptin levels (45). Data from the Overkalix epidemiological data sets also connect patterns of paternal and grand-paternal nutrition with significant changes in offspring development . Here, periods of low availability of food between the ages of 9 and 12 in males, defined as a 'slow growth period' decreased the mortality risk from cardiovascular disease in their offspring (46, 47). In contrast, paternal and grand-paternal over-nutrition during this same period was associated with increased predisposition to diabetes-related mortality (46, 47). Intergenerational paternal programming has also been reported within experimental animal models. Paternal HFD-induced obesity in male rats results in increased adiposity, impaired glucose tolerance and insulin sensitivity within a second (F2) generation (48). Underlying these changes was a significant decrease in DNA methylation in the paternal testicular germ cells associated with the differential expression of 414 genes and 11 miRNAs (48). More recently, we have shown that offspring cardiovascular dysfunction and impaired renin-angiotensin system homeostasis were programed into a second generation in response to a paternal low protein diet in mice (49).

Identifying the mechanisms of paternal programming

The observation that paternal programming can operate over multiple generations implicates epigenetic transmission of paternal traits as one potential mediator. Epigenetic alterations to the sperm (DNA and histone modifications, RNA populations) have been proposed as one mechanism for transmission of paternal programming effects in the offspring. In mice, global sperm DNA hypomethylation, coupled with reduced testicular expression of the key regulatory methyltransferase genes *Dnmt1* and *Dnmt3L*, have been reported in response to a paternal low protein diet (LPD) (50). Aberrant patterns of sperm DNA methylation have also been observed in response to caloric restriction (41) and dietary insufficiency of key vitamins and minerals such as folate (42). Similarly, obesity has been shown to induce alterations to DNA methylation profiles as well as miRNA populations in sperm of male mice (48). In men, differential sperm DNA methylation profiles and ncRNA profiles have also been observed between obese and lean men (51). With regard to paternal obesity in men, studies have shown hypomethylation of the *IGF2* differentially methylated region in offspring leukocytes at birth (52). Furthermore, significant hypomethylation of other imprinted genes including *MEST*, *PEG3* and *NNAT* were

found in offspring of obese fathers (52). Due to the involvement of these genes in growth and metabolic regulation, their differential DNA methylation could be one mechanism linking paternal obesity with altered offspring growth and metabolism. Separately, infertility has been associated with differential sperm DNA methylation (53), histone distribution (54) and RNA content (55). Interestingly, mature sperm contain several different populations of RNA, both within the nucleus and the mitochondria (56). Sperm RNAs are detectible within the fertilised oocyte and can contribute to early embryonic development (57). The significance of sperm RNAs in programming offspring development is exemplified through the observation that the injection of sperm tsRNA fragments isolated from dietary-induced obese mice into control zygotes is able to programme the long-term metabolic ill health in the offspring (58).

Changes to dietary status not only induces change to the composition of the sperm, it can also impact the non-sperm fraction of the semen, the seminal plasma. The role of the seminal plasma in modulating the maternal inflammatory and immune status during the periconception period has been reviewed in detail recently (22, 59). However, a relatively unexplored connection is between the seminal plasma microbiome and paternal reproductive fitness. The mammalian microbiome consists of anywhere between 10 to 100 trillion microorganisms and functions in a symbiotic relationship with its host (60). Until recently, our view of our microbes has centred on their role in pathogenic processes. However, it is now widely accepted that our body's microbiota is central in many developmental, physiological, metabolic and even psychological areas of everyday life. Our bodies possess many different and diverse bacterial populations including our skin, gut and oral microbiomes. Due to modern advances in sequencing capabilities, we are beginning to understand the association between our microbiota and complex conditions such as inflammatory bowel disease (61) and even obesity (62). The interplay between our microbiota and reproductive health has come to the forefront over the past few years with the discussion regarding the sterility of the intra-uterine environment (63). Gaining a better insight into the parental interplay between the maternal reproductive tract and the developing fetus is critical for developing new biomarkers for gestational well-being and both maternal and offspring long-term health. Initial studies into the seminal microbiome focused on detection of pathogenic bacterial species, using comparatively simple techniques such as microscopy and RT-qPCR. In some of the earlier studies, negative associations between the levels of *Anaerococcus* and semen quality were reported (64). In a separate study, semen samples identified as 'normal' within a clinical setting were populated predominantly with *Lactobacillus*, while samples of 'low quality' displayed a predominance of *Prevotella* (65).

One influence of bacteria on male reproduction stems from the toxic effects of inflammatory cytokines or reactive oxygen species produced by them within the male reproductive tract (66). In addition, bacteria may also bind directly to the sperm, influencing motility or inducing apoptosis (67). Prebiotics supplementation in both human and animal models has been shown to influence seminal plasma composition and sperm quality. In obese mice, supplementation with *Lactobacillus rhamnosus* PB01 (DSM 14870) improved sperm kinetics (68). The authors observed increased testosterone levels and sperm with higher velocity and motility in supplemented obese males than non-supplemented obese males (68). In men, increased sperm motility, reduced sperm DNA fragmentation and intracellular H_20_2 levels have also been reported following *Lactobacillus rhamnosu*s CECT8361 and *Bifidobacterium longum* CECT7347 supplementation in asthenozoospermic males (69).

Not only can the seminal microbiome influence male reproductive health, unprotected sexual intercourse can result in the exchange of microbes between partners, suggesting that each partner's reproductive microbiota can affect that of the other. Factors such as frequency of sexual intercourse and number of partners can all be related to the vaginal microbiota and incidences of bacterial vaginosis (70, 71). Therefore, it is conceivable that the male's metabolic status at the time of conception could influence his seminal microbiome, which in turn influence the female reproductive microbiota. As the female reproductive microbiota is directly related to that of the neonate (20), this offers a novel mode of paternal programming of offspring metabolic health. However, such direct demonstration of seminal microbiota paternal programming has yet to be demonstrated.

Conclusions and future perspectives

It is now widely recognised that a connection between sub-optimal *in utero* development and long-term offspring ill-health exists. Despite this wealth of knowledge, much less attention has been given to the influence of the father's lifestyle on the health of his offspring. However, it is becoming increasingly apparent that a father's nutritional status at the time of conception can influence post-fertilisation development through a range of mechanisms (see Figure 1). Sperm quality and functionality can be influenced by factors such as obesity and the associated hormonal imbalances. New sequencing approaches have revealed the epigenetic complexity of the sperm, revealing how sperm can regulate the first few cell cycles of the preimplantation embryo (72). Separately, studies have revealed the role of the seminal plasma in modulating the maternal reproductive tract vascular and immune systems, preparing the uterus for the

implanting embryo (59). With new analyses indicating the seminal microbiome may also influence post-fertilisation development, our understating of this male reproductive component is extending from from just a simple supportive medium for the sperm during their transit through the female reproductive tract, to a central mediator in paternal programming. As our understanding of the interplay between our physiology and our microbiomes increases, modification of our reproductive fitness through changes in our microbiota may open up a new area of personalised reproductive medicine.

Looking forward, there is a clear need to define the associations between other aspects of paternal lifestyle with his nutrition and fertility. Sperm quality is a fundamental component for a successful outcome in ART practices. However, there is a still a heavy reliance on factors such as sperm morphology and motility in guiding practitioners to select single sperm in procedures such as intra cytoplasmic sperm injection (ICSI). As such, a more detailed understanding of what cellular constituents make a 'good quality sperm' are clearly needed. Furthermore, many ART practices are conducted within a seminal plasma-free environment, precluding the normal interaction that occurs between the seminal plasma and the female reproductive tract during natural conception. The impact of removing such interactions for ongoing pregnancy and child health remain to be defined (59). Finally, factors such as advancing paternal age, and the changes in metabolic status that accompany male ageing, have received limited attention until recently. However, connections between paternal age and offspring well-being are becoming more evident (73). We believe that gaining a fuller understanding of how modern lifestyle factors influence paternal metabolic status, sperm quality, embryo development and offspring health is of fundamental significance for ensuring life-long health and well-being of his offspring.

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Conflicts of interest

The authors have no conflicts of interest to declare.

Authors contributions

All authors were involved in the conception, design, preparation and proofing of this paper.

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Figure 1: Outline of key mechanisms linking paternal metabolic status at the time of conception to post-fertilisation development and offspring health. The seminal microbiome has been associated with semen quality and may influence the post-fertilisation maternal uterine microbiome. Seminal plasma cytokines and signalling molecules, such as transforming growth factor-beta, interact with the maternal reproductive tract, priming the immune system and preparing the uterine tissue for the implanting embryo. Changes in epigenetic status of the sperm provide one mechanism capable to propagating paternal influences over multiple generations. Histones have been shown to be located at key pluripotency genes within the paternal genome and have been shown to contribute to the zygotic histone pool after fertilisation. More recently, sperm-borne RNAs (e.g. ncRNA, miRNA, tsRNA) have been shown to be capable of programming offspring metabolic health separate to the genomic content of the sperm. Sperm, as fully differentiated cells, possess high levels of DNA methylation. Changes in sperm DNA methylation can be indicative of a perturbed testicular environment and reduced male fertility.