COMMENTARY

The remarkable legacy of a father's diet on the health of his offspring

or

The gift of fatherhood comes in two parts

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In times past, reproduction was a relatively simple concept, essentially the coming together of sperm and egg and the mixing of paternal and maternal chromosomes to form the new embryonic genome. This would drive the developmental programme, morphogenesis and ultimately the emergence of a new individual. Then came the complication that environment could contribute to the story of reproduction, adding a non-genomic twist to the origins of offspring phenotype. This broadly reflected the recognition of the Barker hypothesis (more recently known as the Developmental Origins of Health and Disease; DOHaD) that maternal factors such as poor diet and physiological condition could adversely influence pregnancy and contribute to offspring risk of cardiometabolic disease in adulthood (1). Subsequently, evidence pointed to the period around conception, notably gamete maturation and early embryogenesis, as a key window when environment may perturb or modify the reproductive process through epigenetic, cellular and physiological mechanisms with DOHaD consequences (2, 3). Moreover, such environmental interactions could happen in fathers as well as mothers and be transmitted at coitus (2, 3). What emerges is the sobering paradigm that parental lifestyle criteria from periconception onwards may have an enduring legacy across the lifespan on offspring health, an influence of sufficient clinical importance to prompt a recent call for preconception health for both partners before pregnancy (4). In the current issue of PNAS, Watkins et al (5) report on paternal programming of offspring disease in a mouse model of low protein diet (LPD) under-nutrition and show, through an elegant experimental design, that paternal sperm and seminal plasma each exert specific yet coordinated pathways by which fathers influence the wellbeing of their progeny. Recognising this duality is important both in devising ways to prevent disease risk and also because in reproductive technologies to overcome human infertility and promote domestic animal production, seminal plasma is either absent or highly diluted.

The paternal LPD model

In the Watkins et al study (5), LPD treatment is limited to just the period of spermatogenesis versus control normal protein diet (NPD) and leads to offspring (fed normal diet) with disturbed metabolic health including increased mass and adiposity, glucose intolerance, a liver gene expression profile suggestive of non-alcoholic fatty liver disease (NAFLD) and an altered gut microbiome. Some of these disease-related outcomes were also reported in previous studies from the group using this model together with cardiovascular defects (hypotension; smaller hearts) (6) and perturbed skeletal development with reduced bone mineral density (7). The adverse programming is detectable through embryo and fetal/placental periods (7) and originates through alterations in both sperm and seminal plasma, as discussed below. Previously, male obesity has been shown to alter concurrently both sperm and seminal plasma composition (8) and past work has established paternal offspring metabolic phenotype to be altered through either sperm or seminal plasma pathways. The major advance by Watkins et al (5) is to define sperm- and seminal plasma-specific effects on offspring within the same study. They use an elegant four-way experimental design combining artificial insemination for sperm provision (LPD or NPD) with vasectomised male mating for seminal plasma provision (LPD or NPD) to 'mix and match' these factors and define what exactly they each contribute.

The sperm pathway of developmental programming

Paternal factors such as over-nutrition and obesity, ageing and infertility have been shown previously to affect sperm DNA integrity, epigenome and RNAs including sperm transcripts (discussed (5, 8)). This leads to a loss in embryo potential and long-term metabolic consequences (discussed (3)). Paternally programmed effects on next generation health appear mediated through sperm epigenome changes (9, 10). The Watkins et al study (5) is consistent and shows the global nature of epigenetic change mediated through paternal LPD with comparative DNA hypomethylation on all sperm chromosomes together with changes in testicular morphology and expression of

epigenetic regulators such as select DNA methyltransferases and folate cycle enzymes. Moreover, the altered sperm epigenome and sperm transcript analysis demonstrated some consistency with gene expression changes occurring in offspring heart tissue (6).

Watkins et al (5) propose that sperm epigenome changes may be mediated through dietary disturbance of the folate cycle capacity to supply methyl groups for DNA methylation as has been shown following maternal LPD treatment (11). Also, null mutation of folate cycle enzyme *Mthfr* causes similar perturbation in sperm epigenome and testicular morphology in a strain-dependent manner (12). It will be interesting to establish the links between paternal diet and sperm epigenetic change and direct consequences on early development. Paternal LPD has been shown to alter expression of signal pathway regulators in the preimplantation embryo, possibly an early step in developmental programming (7). Thus, do LPD sperm alter the RNA pool in the zygote to affect early embryo expression, as shown following male obesity (9)?

The seminal plasma pathway of developmental programming

Seminal fluid consists of secretions from the male accessory glands, namely the prostate, seminal vesicle and bulbo-urethral glands, and also partially from the epididymis (13, 14). The notion that seminal plasma may be a conduit for paternal-maternal communication in reproduction has some appeal and multiple roles and pathways may be involved. The traditional view is that seminal plasma acts to protect sperm integrity and survival by providing nutrients and regulators for sperm maturation. It also activates the acute inflammatory response in the uterus and cervix at coitus to protect against pathogenesis. Further, it promotes the availability of embryotrophic factors such as Lif and Csf2 to support implantation and embryo/fetal development and to provide immune tolerance against paternal antigens (discussed in (5) (13)). Cogent evidence that seminal plasma mediated a more profound legacy came from mating mice after seminal vesicle gland excision (15).

This caused impaired fertility, loss of maternal tract expression of embryotrophic cytokines, placental hypertrophy and postnatal overgrowth and metabolic disturbance mainly in males with increased adiposity, hypertension and glucose intolerance (15). These long-term outcomes could include indirect effects on the unprotected sperm but also direct effects of seminal plasma on the maternal tract.

The seminal plasma cytokine profile in response to paternal LPD was not changed in the Watkins et al study (5), however the uterine pro-inflammatory cytokines and chemokines at 3.5 days post coitus were reduced, together with reduced expression of prostaglandin synthesis genes and reduced uterine blood vessel compliment, all suggesting an altered immunomodulatory outcome in the maternal environment.

The concept that seminal plasma may promote long-term changes in offspring phenotype mediated through paternal diet and physiology is attractive since its composition changes rapidly in response to diet, allowing the dialogue to be dynamic and responsive to environmental stimuli (16). Moreover, the nature of compositional changes and how they may mediate influence on maternal and offspring phenotype are diverse, providing complexity in outcomes. Thus, paternal obesity changes hormonal and metabolite composition of mouse seminal plasma including insulin, leptin and estradiol (8) while in both human and mouse, obesity also alters chronic inflammatory modulators such as TNFa and IL6 (17). Seminal plasma may also transmit extracellular vesicles (EVs) in this communication, containing a complex array of proteins, lipids and nucleic acids to signal to target cells. EVs in seminal fluid are associated with post-testicular sperm maturation, sperm motility acquisition and reduction of oxidative stress (14). EVs in human semen have small non-coding RNAs that are thought to convey an immunomodulatory role (18). Might seminal plasma EVs further transmit paternal modulatory signals to influence the developmental programme? Added to this, seminal vesicles also have their own microbiome which can be changed by paternal diet (19). Seminal plasma signalling may also occur through extracellular matrix remodelling of accessory

glands shown in response to excess homocysteine (20). Lastly, different glandular domains contributing to seminal plasma may have differing responses to paternal environment, perhaps with the prostate more susceptible to methyl-deficient diet (21). Collectively, these varied attributes of seminal plasma in terms of production and sensitivity to environmental factors warrant further investigation as a vehicle for paternal developmental programming.

Mix and match and implications

The' mix and match' sperm/seminal plasma experimental strategy of Watkins et al (5) clearly demonstrates paternal dietary effects on offspring development through both conduits. Thus, postnatal growth and metabolic health were affected by paternal LPD through both sperm and seminal plasma routes. A synergistic effect of LPD when transmitted through both sperm and seminal plasma is only evident in some outcomes such as gut microbiome content. What is intriguing is that the worst prognosis for health commonly emerges in response to a mismatch of sperm and seminal plasma origins (one LPD, the other NPD), evident in particular in the gene expression analysis for NAFLD. This suggests a necessary coordination must occur between sperm and seminal plasma signals to influence maternal and offspring phenotype, otherwise mixed messages and confusion would prevail in the developmental programme. We are left with the concerning question that with the rise in use of reproductive technologies to alleviate human infertility and enhance animal production, and where seminal plasma is effectively missing, what developmental confusions may be induced? Use of such technologies in clinical and domestic animal practice is associated with adverse programming of cardiometabolic health of offspring (2, 3). More research on proteomic and metabolomic profiles of seminal plasma (22) and how these change in response to environmental factors may help improve protocols and safety in assisted conception (13).

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