The sperm factor: paternal impact beyond genes

Simone Immler [1,2]

[1] School of Biological Sciences, University of East Anglia, Norwich Research Park, Norwich, NR4 7TJ, UK

Corresponding author: S. Immler – s.immler@uea.ac.uk

Short title: Paternal epigenetic effects

Keywords: transgenerational epigenetics, methylation, chromatin modification, RNA, paternal effects, sperm quality, centriole-centrosome complex, protein-based inheritance, small non-coding RNA, transposable elements, prion

Abstract

The fact that sperm carry more than just the paternal DNA has only been discovered just over a decade ago. With this discovery, the idea that the paternal condition may have direct implications for the fitness of the offspring had to be revisited. While this idea is still highly debated, empirical evidence for paternal effects is accumulating. Male condition not only affects male fertility but also offspring early development and performance later in life. Several factors have been identified as possible carriers of non-genetic information, but we still know little about their origin and function and even less about their causation. I consider four possible non-mutually exclusive adaptive and non-adaptive explanations for the existence of paternal effects in an evolutionary context. In addition, I provide a brief overview of the main non-genetic components found in sperm including DNA methylation, chromatin modifications, RNAs and proteins. I discuss their putative functions and present currently available examples for their role in transferring non-genetic information from the father to the offspring. Finally, I identify some of the most important open questions and present possible future research avenues.

Introduction

The importance of non-genetic factors for the transmission of information from parents to offspring is increasingly recognized (Bonduriansky and Day, 2009; Bonduriansky, 2012; Bondurianksy and Day, 2018). In animals, the relatively bigger size of the female gamete – the egg – and the resulting transfer of many different non-genetic components from the mother to her offspring has led to an early recognition of the role of maternal non-genetic effects in determining offspring phenotype (e.g. Dickerson, 1947; Willham, 1963; Legates, 1972; see also Bernardo, 1996; Mousseau and Fox, 1998; Wade, 1998; Marshall and Uller, 2007 for reviews). In contrast, the small compact size and the highly reduced cytoplasm of the animal male gamete – the sperm – was one of the main reasons for the assumption that paternal condition plays little to no role in determining offspring phenotype. This assumption has been overturned just over decade ago and it is now recognised that sperm contribute more than the paternal haploid genome (Krawetz, 2005). In this review, I provide an overview of the potential non-genetic mechanisms and factors transferred via sperm into the zygote. I discuss the evidence for their effects across generations, their putative causes and potential consequences in an evolutionary context. This is by no means a complete account and only provides small insights into a highly complex and fascinating world, but it may stimulate further research into the many processes that can be summarized as “sperm factor”.

Male condition and sperm phenotype

Male condition is affected by environmental factors such as diet, temperature and social interactions and these effects are often reflected in the characteristics of a male's ejaculate. Nutritional stress is known to negatively affect sperm quality and can lead to an increase in the number of malfunctioning and morphologically abnormal sperm, which in turn may affect male fertilisation success (Gage and Cook, 1994; Merrells *et al.,* 2009; Perry and Rowe, 2010; Tigreros, 2013; Kahrl and Cox, 2015;). Similarly, variation in environmental temperature affects ejaculate traits such as sperm number and sperm morphology in ectotherm insects (Fox *et al.,* 2006) and fish (Breckels and Neff, 2013) but also in endotherm mammals (e.g. Al-Khanaan *et al.,* 2015). Finally, aspects of male social environment such as male:female ratio and the perceived intensity of sperm competition are known to affect sperm numbers (Arnaud *et al.,* 2001; Pilastro *et al.,* 2002; Pizzari *et al.,* 2003), sperm swimming velocity (Burness *et al.,* 2004) and sperm morphology (Crean and Marshall, 2008; Immler *et al.,* 2010). However, while these environmentally induced changes in ejaculate traits are well established, the potential consequences of such changes for the next generation are poorly understood. In order to estimate the importance of paternal effects we need to understand the non-genetic factors carried by sperm and which part of the zygotic development they might affect.

Why do paternal effects exist?

While the evidence for an effect of the paternal condition on the offspring is rapidly mounting (e.g. Curley *et al.,* 2011; Soubry, 2015; Illum *et al.,* 2018 for

review), the evolutionary reason for the existence of paternal effects is less clear. Here below, I discuss four non-mutually exclusive hypotheses that may serve as possible explanations for the transfer of non-genetic information from the father to the offspring.

Paternal effects are non-adaptive

The transfer of non-genetic factors through sperm could be non-adaptive noise caused by physiological processes affecting the epigenetic mechanisms in the male germline in response to changing environmental conditions experienced by the father. Many of the experimental manipulations used to study paternal effects involve a change in the stress level experienced by the male for a defined period during life. Stress generally evokes strong physiological responses, which may negatively affect the germline and with that male reproduction (McGrady, 2009). These negative effects may include an increase in the production of reactive oxygen species (Dickinson and Chang, 2011) and elevated activity of repetitive elements (Capy *et al.,* 2000), both of which jeopardise the integrity of the genome and may increase mutation rates (Maklakov and Immler, 2016). Defense mechanisms of the genome against such mutagenic factors include DNA methylation, chromatin modifications and the production of small RNAs (sRNAs) including Piwi interacting (piRNAs) and microRNAs (miRNAs; Bartel, 2004; Klattenhof and Theurkauf, 2008; Siomi *et al.,* 2011; Ernst *et al.,* 2017). All three factors are known to be involved in mediating the possible effects of selfish genetic elements at the translational and post-translational levels. As a result, relevant epigenetic marks produced in protection of the germline genome may end up

in the mature gametes as relicts by chance rather than for adaptive reasons. At this stage, the non-adaptive hypothesis needs careful testing before we can exclude it with certainty.

Paternal effects as an adaptive response to increase offspring fitness

The transfer of information about the environmental conditions encountered by the parents to their offspring may be beneficial and provide an adaptive advantage to the offspring (Bonduriansky and Day, 2009; Turner, 2009). A mechanism that allows for such a transfer of information without modifying the genome may offer a flexible solution particularly in rapidly changing environments. A recent theoretical study described a positive feedback process where the parental phenotype favoured by environmental conditions gets progressively reinforced in the following generations through a learning mechanism (Xue and Leibler, 2016). Empirical evidence for such dynamics have been reported in *C. elegans* where small RNAs have been shown to be inherited for several generations without further additional stimulation with the help of RNA-dependent RNA polymerases (Rechavi *et al.,* 2011; Ashe *et al.,* 2012; Gu *et al.,* 2012; Rechavi *et al.,* 2014). A recent study in *C. elegans* provided direct evidence for such a feedback loop determining the duration of transgenerational inheritance of small RNAs (Houri-Ze'evi *et al.,* 2016). Similarly, the ability of prions to assume a self-templating fold mechanism (Harvey *et al.,* 2018) suggests that these have the potential to maintain themselves in a self-regulating manner over many generations. Such genome-independent systems could be a way to memorise past conditions and transfer relevant information across generations for swift adjustments to

slow or rapid environmental changes despite the rigidity of the underlying genome.

Paternal effects to mediate sexual conflict

The inheritance of a paternal and a maternal genome creates a conflict between males and females over allele expression at heterozygous loci in the offspring (Arnqvist and Rowe, 2005). Epigenetic factors may further contribute to this conflict if they are inherited at an equal rate from both parents, but they may also offer a mechanism to resolve the conflict. Genomic imprinting is an epigenetic mechanism, which determines expression of an allele according to its parental origin (Reik and Walter, 2001). The three main theories proposed for the evolution of genomic imprinting are the kinship theory (Haig, 2000), the sexual antagonism theory (Day and Bonduriansky, 2004; Bonduriansky, 2007) and the maternal-offspring co-adaptation theory (Wolf and Hager, 2006; Wolf and Hager, 2009, all reviewed in Patten *et al.,* 2014). The question at the heart of all three theories is the conflict between the parents over gene expression in their offspring at heterozygous loci. The aspect that varies between the theories is the nature of the involved parties (parent-offspring, male-female or all of them together etc.) and of the resolving mechanism.

Similar to the hypothesis presented for sexual conflict, other genetic conflicts have been proposed as a possible explanation for sperm carrying RNAs (Holman and Price, 2014; Hosken and Hodgson, 2014). These authors suggested that RNAs mediate potential genomic conflicts not only between males and females but also between the diploid male and its haploid sperm,

and among the different sperm within an ejaculate. Given the shear variation of RNAs present within each sperm they possibly cover several of these functions.

Paternal effects to control selfish genetic elements

The genomic conflict arising between the genome and selfish genetic elements may provide another explanation for the evolution of transgenerational epigenetic mechanisms (Holman and Price, 2014). The transfer of defense mechanisms against the detrimental effects of stressful environments from the male germline to the zygote would allow the protection of the zygotic genome during the sensitive stages of early development. The findings of variation in small RNA profiles, methylation patterns and chromatin structure in response to environmental stressors in sperm and the resulting offspring appear to be in line with this idea. However, we still know relatively little about the association between transposable elements (TEs) and epigenetic marks and mechanisms. A recent study in *Arabidopsis thaliana* showed that changes in methylation patterns and increased levels of gene expression were directly associated with *de novo* insertions of TEs in the immediate vicinity of affected genes (Stuart *et al.,* 2016). Whether similar associations exist in the male germline and/or in the zygote is currently not known.

Epigenetic factors and RNAs in the sperm may also derive from segregation distorting alleles that involve the incapacitation/killing of sperm or zygotes carrying alternative alleles (Holman and Price, 2014). This suggestion is

purely hypothetical and needs careful testing. But if such a mechanism exists, it would potentially affect male fertility. An association between male fertility and certain RNAs has been shown in humans but the mechanisms involved are unknown (Jodar *et al.,* 2012).

The hypotheses outlined above for the evolution of genetic imprinting and the role of RNAs as signals among different units may apply to any epigenetic factor transmitted via sperm. Males can undoubtedly benefit from transmitting more than just a genome in their gametes, and the idea that these mechanisms are adaptive is enticing. Testing the non-adaptive alternative is therefore even more important and necessary. It will be exciting to examine the different hypotheses and understand more about the evolutionary dynamics involved. This should be increasingly possible with the steadily improving methods available in genomics, transcriptomics and proteomics.

Which non-genetic components does a sperm transfer to the zygote?

Beside the nuclear genome, sperm are known to contain a range of epigenetic elements, which are transferred into the zygote upon fertilisation, including chromatin modifications, RNAs and proteins (reviewed in Dadoune, 2009; Carrell, 2012; Casas and Vavouri, 2014; Rando, 2016; Figure 1). Here below, I provide a brief overview of the currently known factors and present examples for the ways these factors might affect processes in the zygote and beyond. I am using the term “epigenetics” in a broad sense and follow Henikoff and Greally’s (2016) definition, where any cellular memory not encoded in the genetic code is included. Genome-carrying cell organelles

such as mitochondria are therefore not included even though these may be inherited through sperm in rare occasions in some organisms, such as insects (Wolff *et al.,* 2012), mammals (Zhao *et al.,* 2004) and birds (Alexander *et al.,* 2015) and regularly in others such as mussels (Sutherland *et al.,* 1998; Zouros, 2000). Even with this relatively restricted definition of the term epigenetic, condition dependent transgenerational effects may be harder to identify than assumed, and some of the aspects that may need further investigation are described in the section *Current challenges and future directions* below.

DNA methylation/acetylation

DNA methylation is probably the most studied epigenetic mark and is assumed to play a major role in the transfer of non-genetic information across generations. DNA methylation in combination with histone modifications (see section below) plays a key role in regulating gene expression in the germ cells and thereby contributes to three key processes: (I) the specification and formation of primordial germ cells, (II) the genome-wide erasure and re-establishment of germline-specific patterns in the embryo and sex-specific patterns during gametogenesis and (III) the establishment of sex-specific patterns typical for mature male and female gametes (reviewed in Allegrucci *et al.,* 2005). Given their key role in governing gene expression throughout development, it is not surprising that paternal condition affects methylation patterns in the offspring. Fathers kept on a high fat diet in Sprague-Dawley rats for example sired daughters with impaired insulin secretion and glucose tolerance. Their female offspring exhibited altered expression in 642

pancreatic islet genes with some of the key genes being hypomethylated (Ng *et al.,* 2010). More generally, environmental changes during early developmental stages seem to have a major impact on germline methylation patterns (see Faulk and Dolinoy, 2011 for review).

The molecular mechanism is based on the binding of a methyl/acetyl group to a DNA molecule, which may affect the transcriptional activity of the underlying gene without changing the genetic code. The percentage of methylation inherited from the father through sperm varies markedly across taxa and may range from fully maternally inherited to largely paternally inherited patterns. In house mice *Mus musculus* (and other mammals), the methylation structure in the developing zygote is re-structured during early embryogenesis following the maternal template and paternal marks are mostly removed (see Daxinger and Whitelaw, 2012 for review). In contrast, in zebrafish *Danio rerio*, the paternal methylation pattern forms the template and the maternal methylation pattern is largely restructured according to the information coming from the father (Potok *et al.,* 2013; Jiang *et al.,* 2013). These taxonomic differences in methylation inheritance are currently not explained and possible evolutionary reasons need to be tested.

RNA families

Sperm contain many families of RNAs, which may be transferred into the zygote during fertilisation and may therefore affect processes involved during early embryogenesis (Dadoune, 2009). These RNA families include messenger RNAs (mRNA; Alcivar *et al.,* 1989; Ostermeier *et al.,* 2002; Yang

et al., 2009; Bonache *et al.,* 2012), micro RNAs (miRNAs; e.g. Krawetz *et al.,* 2011), Piwi interacting RNAs (piRNAs; e.g. Krawetz *et al.,* 2011), transfer RNA derived small RNAs (tRNAs; e.g. Peng *et al.,* 2012) and a number of other to date un-specified RNA families. mRNAs are a large group of different molecules that are the direct result of gene transcription and are therefore also known as “coding” RNAs. The mRNA content in sperm is relatively low compared to any other cell type, and their origin (i.e. pre- versus postmeiotic) and role need further investigation.

The three remaining families (i.e. miRNAs, piRNAs and tRNAs) belong to the group of “small non-coding” RNAs (sRNAs) as they are transcribed from non-coding regions of the genome, and for many, their origin and function is still unknown. miRNAs are short (about 22-nucleotides) molecules that are involved in RNA silencing and regulation of gene expression at the transcriptional and post-transcriptional stages (Bartel, 2004). They may mediate the activity of selfish genetic elements by triggering small interfering RNAs (siRNAs) in a highly specialised and pathway specific manner (Creasey *et al.,* 2014). Similarly, piRNAS (21-32 nucleotides) in the germline are involved in the silencing of selfish DNA elements and the maintenance of DNA integrity through the formation of RNA-protein complexes that act at the transcriptional and post-transcriptional levels (Klattenhof and Theurkauf, 2008; Siomi *et al.,* 2011; Ernst *et al.,* 2017). However, the exact mechanisms and origins of piRNAs are currently elusive. tRNAs (sometimes also referred to as tsRNAs) may vary in length (from 20 nucleotides into the range of piRNAs) and have been assumed to be the result of transmitter RNA

degradation until they were clearly identified as a distinct group of small non-coding RNAs (Lee *et al.,* 2009). Observations in house mice *M. musculus* suggested that in testicular sperm, the tRNA content is low but increases with maturation through the fusion with epididysomes (Sharma *et al.,* 2016). The same study also reported that the function of these tRNAs is to repress genes associated with the selfish element MERVL active in preimplantation embryos.

The total amount of RNA molecules transferred through sperm is vanishingly small compared to the RNAs present in the egg. Nevertheless, several families of RNAs have been reported to be involved in non-genetic inheritance of paternal conditions across generations. miRNAs and piRNAS were differentially expressed in the sperm of male house mice *M. musculus* exposed to traumatic stress during the juvenile life stage compared to sperm of control male mice (Gapp 2014). The injection of these differentially expressed RNAs into early zygotes lead to similar offspring phenotypes as those observed in the experiments using traumatised males as fathers. Furthermore, miRNAs were involved in the transmission of chronic stress responses experimentally evoked in adult male mice to their offspring (Rodgers *et al.,* 2013). The precise role of tRNAs needs further investigation but they seem to affect gene expression during early embryo development (Sharma *et al.,* 2016).

Proteins

Sperm are composed of a wide range of proteins located on the sperm surface, in the acrosome (where present), in and around the nucleus and even in the flagellum. The sperm proteosome as a whole has been analysed with respect to human infertility and 20 proteins have been identified to be associated with fertility issues (Lefievre *et al.,* 2003; Pixton *et al.,* 2004; Rawe *et al.,* 2008). A similar study in the house mouse *M. musculus* shortlisted 132 proteins that may affect fertility, some of which seem to be evolutionarily preserved across taxonomic groups (Chu *et al.,* 2006). These findings suggest a potential major role for proteins in transgenerational epigenetics.

In fact, in non-rodent mammalian fertilisation, the centriole-centrosome is inherited through the sperm and acts as a template for all subsequent cell divisions from early embryogenesis into adulthood. Any malformations of this complex result in severe infertility due to disruption or insufficiency during mitotic divisions and may hence cause developmental problems anywhere from interrupting the first mitotic divisions to causing embryonic malformations (Schatten and Sun, 2013). The centriole-centrosome complex likely varies in its shape and therefore function also among fertile males, and these more subtle variations may contribute to the fitness and performance of the offspring in the next generation.

In a recent review, Harvey *et al.* (2018) proposed that prions are ideal candidates for non-genetic transgenerational inheritance due to their conformational flexibility and their ability to transform into self-templating folds, which allows them to proliferate independently even across generations.

Prions are considerably more stable during meiotic processes compared to other epigenetic factors experiencing major re-structuring (Cox, 1965; Young and Cox, 1971). The independence and stability of prions may imply that protein-based transgenerational inheritance could be important but the idea needs careful testing.

Histone modifications

Although histone modifications could be regarded as part of the sperm proteome, I discuss them separately as they have received a lot of attention in the context of trans-generational epigenetics. Modifications of the histones are assumed to affect gene expression and therefore may play a key role in gene regulation (e.g. Kouzarides, 2007). Gene regulation is particularly important during the early stages of development and any marks inherited from the father may contribute to embryonic gene expression – with potential effects later on in life. In mammalian sperm, 90 (in humans) to 95% (in house mice) of histones are replaced by protamines during spermatogenesis, and the remaining histones may undergo post-translational modifications affecting gene expression at these loci (Luense *et al.,* 2016). These post-translational modifications may regulate gene expression during spermatogenesis and during early embryo development (Brykczynska *et al.,* 2010; Hammoud *et al.,* 2011; Erkek *et al.,* 2013; Brunner *et al.,* 2014). In human sperm, histone modifications appear to be particularly enriched around developmental loci. Dimethylated lysine 4 on histone H3 (H3K4me2) for example, is found at promoter loci, whereas H3K4me3 is found in large clusters of paternally expressed imprinted genes, miRNAs and HOX genes (Hammoud *et al.,*

2009). These patterns also seem to hold for the zebrafish *Danio rerio*, where sperm retain the histones and lack protamines altogether, but chromatin markers such as permissive H3K4me3 with or without repressive H3K9me3 or H3K27me3 are associated with developmental loci (Lindeman *et al.,* 2011). A study manipulating the dietary conditions in male house mice observed differential gene expression in the next generation and found a consistent decrease in H3K27me3 at the promoter of monoamine oxidase in sperm of low-protein diet males compared to control males (Carone *et al.,* 2010).

Current challenges and future directions

The study of paternal epigenetic effects inherited across generations is still in its early days and many fundamental questions are currently unanswered. The many unfilled gaps and fundamental unknowns put limitations to our ability to summarise the relative importance, prevalence, and/or impact of each of the factors discussed. It may be worth identifying some of the key aspects that we should focus on in the near future.

The term “sperm factor” may be somewhat misleading in being an oversimplification of what is clearly a varied set of highly complex factors. One of the questions is therefore: How are the different mechanisms linked? Understanding whether the different epigenetic components act independently, complementarily, additively, or interactively and how these interactions and the resulting effects may be context-dependent are some of the challenges we are currently facing. The interaction between some of the factors such as the tight linkage between DNA methylation and histone

modifications for the regulation of gene expression during proliferation and differentiation of the germline is relatively well understood. In contrast, other factors are still largely a black box (piRNAs), and many have not even been properly identified yet (other small RNAs). Carefully designed experiments combined with the latest –omics technology may be a valuable way to gain insights into what are clearly highly complex processes.

Another currently open question is whether the non-genetic transfer of information in sperm is truly “non-genetic” or whether there is a causal connection between the non-genetic information and the underlying genome. Non-genetic factors may fall into one of three possible categories: (I) independent of sequence variation, (II) partially dependent on sequence variation, and (III) completely dependent on sequence variation (based on epiallelic variation as proposed by Richards, 2006). An additional aspect that needs to be considered is whether the transfer of information is based (A) purely on transmitted genes or (B) on a combination of transmitted genes and non-genetic material. In case III, all the observed variation should be explained by focusing exclusively on sequence variation and the distinction between scenario A and B is not necessary. However, in cases I and II, sequence variation will not explain everything as non-genetic material may be generated independently and add variation through non-genetic mutations occurring between transcription events. Performing experimental manipulations of paternally experienced environmental conditions in combination with long-read DNA sequencing, RNA sequencing of different

RNA families, ChIP sequencing and bisulfite sequencing is not an easy but a promising way forward to answer these questions.

The importance of the relative timing and duration of changes in environmental conditions experienced by a male to affect the following generation(s) is still poorly understood. In mammals (and probably most other taxa), early embryo development is a particularly sensitive period and methylation patterns and histone modifications are strongly affected by environmental conditions during this time (reviewed in Faulk and Dolinoy, 2011). However, effects across generations have also been shown in studies where males were exposed to stressful environments as juveniles before sexual maturity (e.g. Gapp *et al.,* 2014), during adulthood (e.g. Carone *et al.,* 2010) or both (e.g. Rodgers *et al.,* 2013). It would be interesting to understand, which epigenetic factors are mostly affected by environmental conditions in the male germline during each of these life stages and how strong the observed transgenerational effects are relative to each other.

Of particular relevance for the fields of ecology and evolution is the question about the stability of epigenetic alterations. While some epigenetic marks are stable and conserved even across taxa (Provataris *et al*., 2018), others are seemingly more apt to change. Having said that, even sRNAs can be transferred across many generations without further stimulation in a self-regulating process (Rechavi *et al.,* 2014) suggesting that such systems may provide a reliable way to memorise environmental conditions. Understanding

the flexibility and stability of epigenetic mechanisms is important to fully assess their relative contribution to inheritance.

Finally, ejaculates generally consist of more than just sperm, and we know that the content of seminal fluids may have severe effects on female fitness (Chapman *et al.,* 1995; Wolfner, 2002), and also on their offspring (Chapman *et al.,* 2001; Crean *et al.,* 2014; Crean *et al.,* 2016). Controlling for such effects and disentangling factors carried by sperm from factors in the seminal fluid will be imperative when studying the various mechanisms.

In summary, non-genetic factors transferred through the sperm into the zygote are very likely to affect the resulting generation(s) and this in itself is a very important insight. We now need to understand, which mechanisms contribute to this transfer of information and how and what the true purpose of non-genetic information transferred in sperm across generations is. With a great range of novel tools becoming available and increasingly affordable we should be able to address these important questions.

Acknowledgements

I thank F. Spagopoulou and M. Lind for giving me the opportunity to write this review and to A. S.-J. Suh for inspiring discussions and four anonymous reviewers for valuable comments on earlier versions of this manuscript. I was supported by grants from the European Research Council (HAPSELA336633), Human Frontier Science Program (R0025/2015) and the Knut and Alice Wallenberg Foundation.

Conflict of interest

The authors declare no conflict of interest.

References

Al-Khanaan A, König S, Brügemann K (2015). Effects of heat stress on semen characteristics of Holstein bulls estimated on a continuous phenotypic and genetic scale. *Livestock Sci* **177:** 15-24.

Alcivar AA, Hake LE, Millette CF, Trasler JM, Hecht NB (1989). Mitochodrial gene expression in male germ cells of the mouse. *Dev Biol* **135:** 263-271.

Alexander M, W HSY, Molak M, Barnett R, Carlborg Ö, Dorshorst B *et al* (2015). Mitogenomic analysis of a 50-generation chicken pedigree reveals a rapid rate of mitochondrial evolution and evidence for paternal mtDNA inheritance. *Biol Lett* **11:** 20150561.

Allegrucci C, Thurston A, Lucas E, Young LJ (2005). Epigenetics and the germline. *Reproduction* **129:** 137-149.

Arnaud L, Haubruge E, Gage MJG (2001). Sperm size and number variation in the red flour beetle. *Zool J Linn Soc* **133:** 369-375.

Arnqvist G, Rowe L (2005). *Sexual Conflict*. University Press: Princeton.

Ashe A, Sapetschnig A, Weick E-M, Micthell J, Bagjin MP, Cording AC *et al* (2012). piRNAs can trigger a multigenerational epigenetic memory in the germline of *C. elegans*. *Cell* **150:** 88-99.

Bartel DP (2004). MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* **116:** 281-297.

Bernardo J (1996). Maternal effects in animal ecology. *Am Zool* **36:** 83-105.

Bonache S, Mata A, Ramos MD, Bassas L, Larriba S (2012). Sperm gene expression profile is related to pregnancy rate after insemination and is predictive of low fecundity in normozoospermic men. *Hum Reprod* **27:** 1556-1567.

Bonduriansky R (2007). The genetic architecture of sexual dimorphism: the potential roles of genomic imprinting and condition dependence. In: Fairbairn DJ, Blanckenhorn WU and Székely T (eds) *Sex, Size, and Gender Roles: Evolutionary Studies of Sexual Dimorphism*. Oxford University Press: Oxford, pp 176-184.

Bonduriansky R (2012). Rethinking heredity, again. *Trends Ecol Evol* **27:** 330-336.

Bonduriansky R, Day T (2009). Nongenetic inheritance and its evolutionary implications. *Ann Rev Ecol Evol Syst* **40:** 103-125.

Bonduriansky R, Day T (2018). *Extended Heredity: A New Understanding of Inheritance and Evolution*. Princeton University Press.

Breckels RD, Neff BD (2013). The effects of elevated temperature on the sexual traits, immunology and survivorship of a tropical ectotherm. *J Evol Biol* **216:** 2658-2664.

Brunner AM, Nanni P, Mansuy IM (2014). Epigenetic marking of sperm by post-translational modification of histones and protamines. *Epigenet Chromatin* **7:** 2.

Brykczynska U, Hisano M, Erkek S, Ramos L, Oakeley EJ, Roloff TC *et al* (2010). Repressive and active histone methylation mark distinct promoters in human and mouse spermatozoa. *Nature Struct Mol Biol* **17**: 679-687.

Burness G, Casselman SJ, Schulte-Hostedde AI, Moyes CD, Montgomerie R (2004). Sperm swimming speed and energetics vary with sperm competition risk in bluegill (*Lepomis macrochirus*). *Behav Ecol Sociobiol* **56:** 65-70.

Capy P, Gasperi G, Bièmont C, Bazin C (2000). Stress and transposable elements: co-evolution or useful parasites? *Heredity* **85:** 101-106.

Carone BR, Fauquier L, Habib N, Shea JM, Hart CE, Li R *et al* (2010). Paternally induced transgenerational environmental reprogramming of metabolic gene expression in mammals. *Cell* **143:** 1084-1096.

Carrell DT (2012). Epigenetics of the male gamete. *Fertil Steril* **97:** 267-274.

Casas E, Vavouri T (2014). Sperm epigenomics: challenges and opportunities. *Front Genet* **5:** 330.

Chapman T, Herndon LA, Heifetz Y, Partridge L, Wolfner MF (2001). The Acp26Aa seminal fluid protein is a modulator of early hatchability in *Drosophila melanogaster*. *Proc R Soc Lond B* **268:** 1647-1654.

Chapman T, Liddle LF, Wolfner MF, Partridge L (1995). Cost of mating in *Drosophila melanogaster* females is mediated by male acessory-gland products. *Nature* **373:** 241-244.

Chu DS, Liu HL, Nix P, Wu TF, Ralston EJ, Yates III JR *et al* (2006). Sperm chromatin proteomics identifies evolutionary conserved fertility factors. *Nature* **443:** 101-105.

Cox BS (1965). A cytoplasmic suppressor of super-suppressor in yeast. *Heredity* **20:** 505-521.

Crean AJ, Adler MI, Bonduriansky R (2016). Seminal fluid and mate choice: new predictions. *Trends Ecol Evol* **31:** 253-255.

Crean AJ, Kopps A, Bonduriansky R (2014). Revisiting telegony: Offspring inherit an acquired characteristic of their mother's previous mate. *Ecol Lett* **17:** 1545-1552.

Crean AJ, Marshall DJ (2008). Gamete plasticity in a broadcast spawning marine invertebrate. *Proc Nat Acad Scie USA* **105:** 13508-13513

Creasey KM, Zhai J, Borges F, Van Ex F, Regulski M, Meyers BC *et al* (2014). miRNAs trigger widespread epigenetically activated siRNAs from transposons in *Arabidopsis*. *Nature* **508:** 411-415.

Curley JP, Mashood R, Champagne FA (2011). Epigenetics and the origins of paternal effecst. *Horm Behav* **59:** 306-314.

Dadoune J-P (2009). Spermatozoal RNAs: What about their functions? *Microscopy Res Tech* **72:** 536-551.

Daxinger L, Whitelaw E (2012). Understanding transgenerational epigenetic inheritance via the gametes in mammals. *Nature Rev Genet* **13:** 153-162.

Day T, Bonduriansky R (2004). Intralocus sexual conflict can drive the evolution of genomic imprinting. *Genetics* **167:** 1537-1546.

Dickerson GE (1947). Composition of hog carcasses as influenced by heritable differences in rate and economy of gain. *Res Bull Iowa Agric Exp Station* **354:** 489–524.

Dickinson BC, Chang CJ (2011). Chemistry and biology of reactive oxygen species in signaing or stress responses. *Nature Chem Biol* **7:** 504-511.

Erkek S, Hisano M, Liang C-Y, Gill M, Murr R, Dieker J *et al* (2013). Molecular determinants of nucleosome retention at CpG-rich sequences in mouse spermatozoa. *Nat Struct Mol Biol* **20:** 868-875.

Ernst C, Odom DT, Kutter C (2017). The emergence of piRNAs against transposon invasion to preserve mammalian genome integrity. *Nature Comms* **8:** 1411.

Faulk C, Dolinoy DC (2011). Timing is everything. *Epigenetics* **6:** 791-797.

Fox CW, Stillwell RC, Wallin WG, Hitchcock LJ (2006). Temperature and host species affect nuptial gift size in a seed-feeding beetle. *Func Ecol* **20:** 1003-1011.

Gage MJG, Cook PA (1994). Sperm size or numbers - effects of nutritional stress upon eupyrene and apyrene sperm production strategies in the moth *Plodia interpunctella* (Lepidoptera, Pyralidae). *Func Ecol* **8:** 594-599.

Gapp K, Jawaid A, Sarkies P, Bohacek J, Pelczar P, Prados J *et al* (2014). Implication of sperm RNAs in transgenerational inheritance of the effects of early trauma in mice. *Nature Neurosci* **17:** 667-669.

Gu SG, Pak J, Guang S, Maniar JM, Kennedy S, Fire A (2012). Amplification of siRNA in Caenorhabditis elegans generates a transgenerational sequence-targeted histone H3 lysine 9 methylation footprint. *Nature Genet* **44:** 157-164.

Haig D (2000). The kinship theory of genomic imprinting. *Ann Rev Ecol Syst* **31:** 9-32.

Hammoud SS, Nicx DA, Zhang H, Purwar J, Carrell DT, Cairns BR (2009). Distinctive chromatin in human sperm packages genes for embryo development. *Nature* **460:** 473-479.

Hammoud SS, Nix DA, Hammoud AO, Gibson M, Cairns BR, Carrell DT (2011). Genome-wide analysis identifies changes in histone retention and epigenetic modifications at developmental and imprinted gene loci in the sperm of infertile men. *Hum Reprod* **26**: 2558-2569.

Harvey ZH, Chen Y, Jarosz DF (2018). Protein-based inheritance: epigenetics beyond the chromosome. *Mol Cell* **69:** in press.

Henikoff S, Greally JM (2016). Epigenetics, cellular memory and gene regulation. *Curr Biol* **26:** R644-R648.

Holman L, Price TAR (2014). Even more functions of sperm RNA: a response to Hosken and Hodgson. *Trends Ecol Evol* **29:** 648-649.

Hosken DJ, Hodgson DJ (2014). Why do sperm carry RNA? Relatedness, conflict, and control. *Trends Ecol Evol* **29:** 451-455.

Houri-Ze'evi L, Korem Y, Sheftel H, Faigenblom L, Toker IA, Dagan Y *et al* (2016). A tunable mechanism determines the duration of the transgenerational small RNA inheritance in *C. elegans*. *Cell* **165:** 88-99.

Illum LRH, Bak ST, Lund S, Nielsen AL (2018). DNAmethylation in epigenetic inheritance of metabolic diseases through the male germ line. *J Mol Endocrinol* **60:** R39-R56.

Immler S, Pryke SR, Birkhead TR, Griffith SC (2010). Pronounced within-individual plasticity in sperm morphometry across social environments. *Evolution* **64:** 1634-1643.

Jodar M, Kalko S, Castillo J, Ballescà J, Oliva R (2012). Differential RNAs in the sperm cells of asthenozoospermic patients. *Hum Reprod* **27:** 1431-1438.

Jiang L, Zhang J, Wang J-J, Wang L, Zhang L, Li G *et al* (2013). Sperm, but not oocyte, DNA methylome is inherited by zebrafish early embryos. *Cell* **153:** 773-784.

Kahrl AF, Cox RM (2015). Diet affects ejaculate traits in a lizard with condition-dependent fertilization success. *Behav Ecol* **26:** 1502-1511.

Klattenhof C, Theurkauf W (2008). Biogenesis and the germline functions of piRNAs. *Development* **135:** 3-9.

Kouzarides T (2007). Chromatin modifications and thir function. *Cell* **128:** 693-705.

Krawetz S (2005). Paternal contribution: New insights and future challenges. *Nature Rev Genet* **6**(8)**:** 633-642.

Krawetz SA, Kruger A, Lalancette C, Tagett R, Anton E, Draghici S *et al* (2011). A survey of small RNAs in human sperm. *Hum Reprod* **26:** 3401-3412.

Lee YS, Shibata Y, Malhotra A, Dutta A (2009). A novel class of small RNAs: tRNA-derived RNA fragments (tRFs). *Gen Develop* **23:** 2639-2649.

Lefievre L, Barratt CL, Harper CV, Conner SJ, Flesch FM, Deeks E *et al* (2003). Physiological and proteomic approaches to studying prefertilization events in the human. *Reprod BioMed Online* **7:** 419-427.

Legates JE (1972). The role of maternal effects in animal breeding. IV. Maternal effects in laboratory species. *J Anim Sci* **35**(6)**:** 1294-1302.

Lindeman L, Andersen I, Reiner A, Li N, Aanes H, Ostrup O *et al* (2011). Prepatterning of developmental gene expression by modified histones before zygotic genome activation. *Dev Cell* **21:** 993-1004.

Luense LJ, Wang X, Schon SB, Weler AH, Shiao EL, Bryant JM *et al* (2016). Comprehensive analysis of histone post-translational modifications in mouse and human male germ cells. *Epigenet Chrom* **9:** 24.

Maklakov AA, Immler S (2016). The expensive germline and the evolution of ageing. *Curr Biol* **26:** R577-R586.

Marshall DJ, Uller T (2007). When is a maternal effect adaptive? *Oikos* **116:** 1957-1963.

McGrady AV (2009). Effects of Psychological Stress on Male Reproduction: A Review. *Arch Androl* **13:** 1-7.

Merrells KJ, Blewett H, Jamieson JA, Taylor CG, Suh M (2009). Relationship between abnormal sperm morphology induced by dietary zinc deficiency and lipid composition in testes of growing rats. *Brit J Nut* **102:** 226-232.

Mousseau TA, Fox CW (1998). The adaptive significance of maternal effects. *Trends Ecol Evol* **13**(10)**:** 403-407.

Ng S-F, Lin RCY, Laybutt DR, Barres R, Owens JA, Morris MJ (2010). Chronic high-fat diet in fathers programs β-cell dysfunction in female rat offspring. *Nature* **467:** 963-966.

Ostermeier GC, Dix DJ, Miller D, Khatri P, Krawetz SA (2002). Spermatozoal RNA profiles of normal fertile men. *Lancet* **360:** 772-777.

Patten MM, Ross L, Curley JP, Queller DC, Bonduriansky R, Wolf JB (2014). The evolution of genomic imprinting: theories, predictions and empirical tests. *Heredity* **113:** 119-128.

Peng H, Shi J, Zhang Y, Zhang H, Liao S, Li W-H *et al* (2012). A novel class of tRNA-derived small RNAs extremely enriched in mature mouse sperm. *Cell Res* **22:** 1609-1612.

Perry JC, Rowe L (2010). Condition-dependent ejaculate size and composition in a ladybird beetle. *Proc R Soc Lond B* **277:** 3639–3647.

Pilastro A, Scaggiante M, Rasotto MB (2002). Individual adjustment of sperm expenditure accords with sperm competition theory. *Proc Nat Acad Sci USA* **99**(15)**:** 9913-9915.

Pixton KL, Deeks ED, Flesch FM, Moseley FL, Bjorndahl L, Ashton PR *et al* (2004). Sperm proteome mapping of a patient who experienced failed fertilization at IVF reveals altered expression of at least 20 proteins compared with fertile donors: case report. *Hum Reprod* **19:** 1438-1447.

Pizzari T, Cornwallis CK, Løvlie H, Birkhead TR (2003). Sophisticated sperm allocation in male fowl. *Nature* **426:** 70-74.

Potok M, Nix D, Parnell T, Cairns B (2013). Reprogramming the maternal zebrafish genome after fertilization to match the paternal methylation pattern. *Cell* **153:** 759-772.

Provataris P, Meuseman K, Niehuis O, Grath S, Misof B (2018). Signatures of DNA methylation across insects suggest reduced DNA methylation levels in Holometabola. *Genome Biol Evol* **10**: 1185–1197.

Rando OJ (2016). Intergenerational transfer of epigenetic information in sperm. *Cold Spring Harb Perspect Med* **6**(5).

Rawe VY, Díaz ES, Abdelmassih R, Wójcik C, Morales P, Sutovsky P *et al* (2008). The role of sperm proteasomes during sperm aster formation

and early zygote development: implications for fertilization failure in humans. *Hum Reprod* **23:** 573-580.

Rechavi O, L Houri-Ze'evi L, S Anava S, WSS Goh WSS, SY Kerk SY, Hannon GJ *et al* (2014). Starvation-induced transgenerational inheritance of small RNAs in C. elegans. *Cell* **158:** 277-287.

Rechavi O, Minevich G, O H (2011). Transgenerational inheritance of an acquired small RNA-based antiviral response in C. elegans. *Cell* **147:** 1248-1256.

Reik W, Walter J (2001). Genomic imprinting: parental influence on the genome. *Nature Rev Genet* **2**(1)**:** 21-32.

Richards EJ (2006). Inherited epigenetic variation — revisiting soft inheritance. *Nature Rev Genet* **7:** 395.

Rodgers AB, Morgan CP, Bronson SI, Revello S, Bale TL (2013). Paternal stress exposure alters sperm microRNA content and reprograms offspring HPA stress axis regulation. *J Neurosci* **33:** 9003-9012.

Schatten H, Sun Q-Y (2013). The role of the sperm centrosome in reproductive fitness. In: Carrell D (ed) *Paternal Influences on Human Reproductive Success*. Cambridge University Press: Cambridge, pp 50-60.

Sharma U, Conine CC, Shea JM, Boskovic A, Derr AG, Bing XY *et al* (2016). Biogenesis and function of tRNA fragments during sperm maturation and fertilization in mammals. *Science* **351:** 391-396.

Siomi MC, Sato K, Pezic D, Aravin AA (2011). PIWI-interacting small RNAs: the vanguard of genome defence. *Nat Rev Mol Cell Biol* **12:** 246-258.

Soubry A (2015). Epigenetic inheritance and evolution: A paternal perspective on dietary influences. *Progr Biophys Mol Biol* **118:** 79-85.

Stuart T, Eichten SR, Cahn J, Karpievitch YV, Borevitz JO, Lister R (2016). Population scale mapping of transposable element diversity reveals links to gene regulation and epigenomic variation. *eLife* **5:** e20777.

Sutherland B, Stewart D, Kenchington ER, Zouros E (1998). The fate of paternal mitochondrial DNA in developing female mussels, Mytilus edulis: implications for the mechanism of doubly uniparental inheritance of mitochondrial DNA. *Genetics* **148:** 341-347.

Tigreros N (2013). Linking nutrition and sexual selection across life stages in a model butterfly system. *Funct Ecol* **27:** 145-154.

Turner BM (2009). Epigenetic responses to environmental change and their evolutionary implications. *Phil Trans R Soc Lond B* **364:** 3403-3418.

Wade MJ (1998). The evolutionary genetics of maternal effects. In: Mousseau TA and Fox CW (eds) *Maternal effects as adaptations*. Oxford University Press: Oxford, pp 5-21.

Willham RL (1963). The covariance between relatives for characters composed of components contributed by related individuals. *Biometrics* **19:** 8-27.

Wolf JB, Hager R (2006). A maternal-offspring coadaptation theory for the evolution of genomic imprinting. *PLoS Biol* **4**: 2238-2243.

Wolf JB, Hager R (2009). Selective abortion and the evolution of genomic imprinting. *J Evol Biol* **22:** 2519-2523.

Wolff JN, Nafisinia M, Sutovsky P, Ballard JWO (2012). Paternal transmission of mitochondrial DNA as an integral part of mitochondrial inheritance in metapopulations of Drosophila simulans. *Heredity* **110**(1)**:** 57-62.

Wolfner MF (2002). The gifts that keep on giving: physiological functions and evolutionary dynamics of male seminal proteins in *Drosophila*. *Heredity* **88:** 85-93.

Xue BK, Leibler S (2016). Evolutionary learning of adaptation to varying environments through a transgenerational feedback. *Proc Nat Acad Sci USA* **113:** 11266-11271.

Yang CC, Lin YS, Hsu CC, Wu SC, Lin EC, Cheng WTK (2009). Identification and sequencing of remnant messenger RNAs found in domestic swine (*Sus scrofa*) fresh ejaculated spermatozoa. *Anim Reprod Sci* **113:** 143-155.

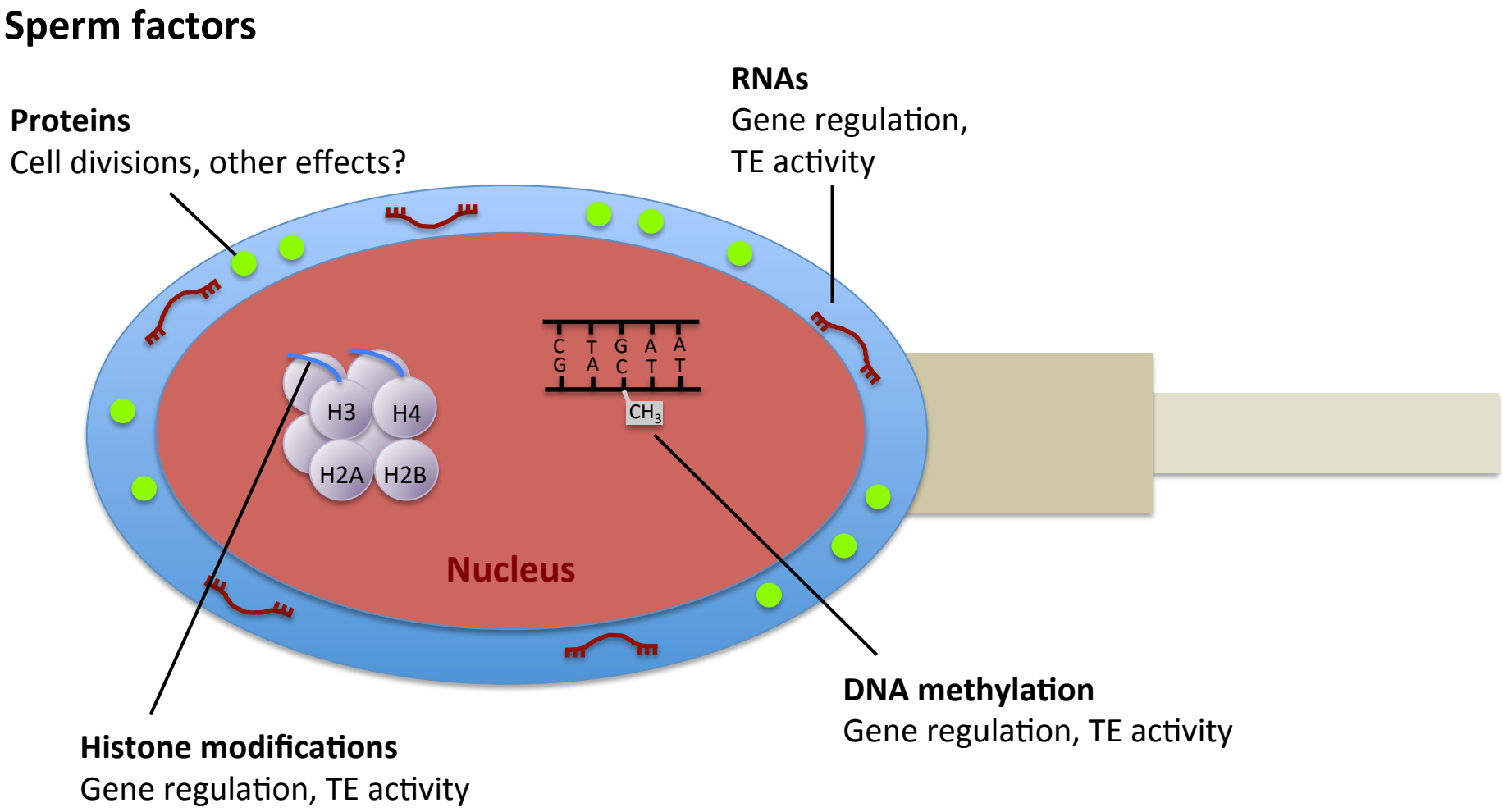
Young CSH, Cox BS (1971). Extrachromosomal elements in a supersuppression system of yeast I. A nuclear gene controlling the inheritance of the extrachromosomal elements. *Heredity* **26:** 413-422.

Zhao X, Li N, Guo, Hu X, Liu Z, Gong G *et al* (2004). Further evidence for paternal inheritance of mitochondrial DNA in the sheep (*Ovis aries*). *Heredity* **93**(4)**:** 399-403.

Zouros E (2000). The exceptional mitochondrial DNA system of the mussel family Mytilidae. *Genes Genet Syst* **75**(6)**:** 313-318.

Figure 1: Illustration of non-genetic components transferred via sperm from the father to the offspring and their putative effects in the offspring. The description of the effects is very general as many of them are currently still poorly understood.

Figure 1


Sperm factors
Proteins
Cell divisions, other effects?
RNAs
Gene regulation,
TE activity
H3
H4
H2A
H2B
C T G A G C A T A T
CH3
Nucleus
Histone modifications
Gene regulation, TE activity
DNA methylation
Gene regulation, TE activity