Received: 27 November 2020 | Revised: 28 December 2020 | Accepted: 10 January 2021

DOI: 10.1111/eci.13492

PERSPECTIVE

WILEY

Sperm, metabolic memory and echoes from Lamarck

Abstract

- There is a widespread misconception that only maternal variables affect *in utero* development.
- Epigenetic markers carried by the spermatozoon are transmitted to the zygote.
- Sperm-born epigenetic factors influence *in utero* development, for various generations.
- Acquired traits of metabolic disease can be inherited by the offspring via the male gamete.
- Health assessment of future fathers is essential to predict the offspring's health.

1 | INTRODUCTION

The importance of environmental variables in utero has been acknowledged for several decades. Clinical evidence regarding the negative impact of maternal alcohol and drug consumption, tobacco and prescription drug use, unhealthy diet and sedentarism, obesity and diabetes, and pollutant and chemical exposures has originated guidelines for pregnant women worldwide to reduce newborn mortality and morbidity rates sharply.[1] However, this evidence also created an incorrect assumption of the uterus as an 'isolated system', whose conditions are uniquely dependent on the woman. However, recent reports highlight that the spermatozoon carries more than just genomic information that will influence the embryo development in the uterus. Notably, epigenetic signatures, such as DNA methylation and small noncoding RNAs (sncRNA), are also carried by spermatozoa and encode acquired traits that may be inherited, hence programming the offspring.

2 | THE LONG WAY OF EPIGENETIC MARKERS FROM TESTIS TO UTERUS

The role of the male gamete in embryonic development has long been neglected, mostly due to a lack of knowledge on the epigenetic markers that can 'survive' in spermatozoon and the zygote. The advances made in the description of the epigenetic mechanisms created theoretical 'dead ends' for the transgenerational inheritance of epigenetic signatures via the male lineage. Among these critical epigenetic points, probably the greatest obstacles were the theories of the 'epigenetic reset' and the 'bottleneck' (Figure 1). After the discovery of the importance of DNA methylation as an epigenetic mechanism to control gene expression, and especially considering that methylation marks are preserved in the semi-conservative replication of DNA, it was thought that germline cells could transmit those methylation patterns to the offspring.[2] However, it was previously demonstrated that germline cells go through several demethylation events to recover totipotency, even after gamete fusion. Moreover, it was known that histones were replaced by protamines during spermatogenesis, thereby erasing any histone modification that could alter the condensation state of chromatin.[3] Besides, according to the 'bottleneck' theory, the huge difference in cytoplasmic volume between the spermatozoon and the oocyte was suggested to hinder any factors carried by the male gamete since it would be too diluted to have any significant impact on the developing embryo.[4]

It was known that environmental variables could affect health outcomes on the offspring, even by the male lineage. Probably the earliest scientific evidence of this phenomenon dates to 2002, to the Överkalix study in northern Sweden.[5] This study, based on historical records of three cohorts (born in 1890, 1905, and 1920) of the remote community, demonstrated that food availability during the slow growth period (SGP) of boys influences the risk for the onset of obesity and diabetes in their grandsons. This work caused controversy due to the absence of a known mechanism that could support the inheritance of acquired traits via the male lineage, and also due to the weight of cultural factors that hampered the scientific relevance of the findings.

Following this study, several human and animal studies further supported the hypothesis that acquired traits could be equally transmitted via maternal and paternal lineage, but the mechanisms involved in the paternal epigenetic inheritance remained largely unknown. It was not before 2008 that a mechanism supporting transgenerational inheritance of acquired traits by sperm was described. One of the

first advancements was the discovery that not all histones in sperm are replaced by protamines.[6] van der Heiden and colleagues further expanded the relevance of the retained histones by injecting human sperm into murine oocytes and showing that the retained histones of sperm chromatin contributed to paternal zygote chromatin.[6] This work highlighted that the epigenetic markers added to retained histones may be inherited by the offspring. However, and contrary to previous studies, it was not clear which traits could be transmitted. More complete evidence emerged from animal models of *in utero* exposure to different stimuli. In 2010, two research groups have independently reported that the administration of the fungicide vinclozolin

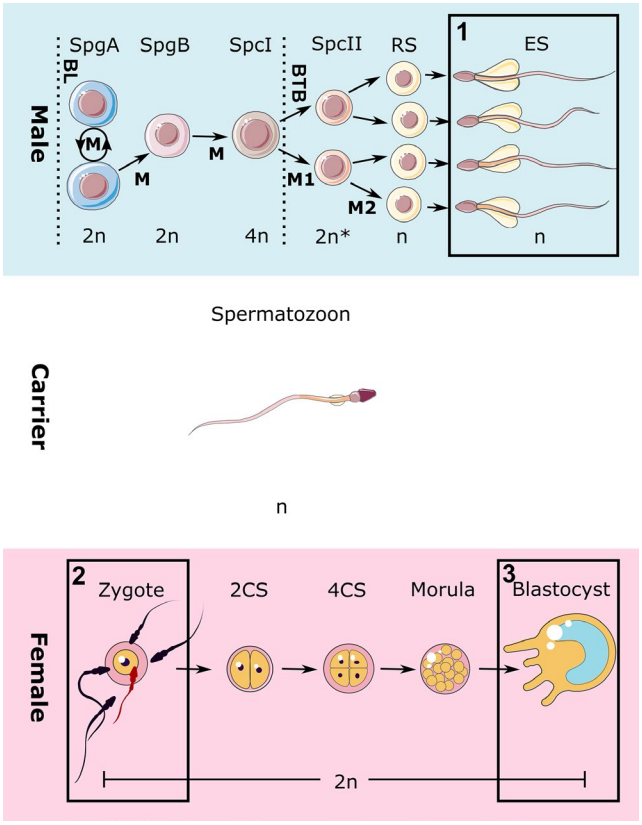

FIGURE 1 Critical barriers to paternal transgenerational epigenetic inheritance. Epigenetic remodelling events constantly modify the epigenetic cargo of male germline cells in the male body, in transit (spermatozoa), and in the female body. However, three major epigenetic events were thought to completely erase all potential epigenetic markers carried by spermatozoa. 1—Histone removal and extensive cytoplasmic reduction. 2—After fertilization: demethylation of paternal DNA and 'bottleneck' effect. 3—Extensive DNA remethylation. Abbreviations: BL—basal lamina; BTB—blood-testis barrier; M—mitosis; M1—meiosis 1; M2—meiosis 2; SpgA—spermatogonia A; SpgB—spermatogonia B; SpcI—primary spermatocyte; SpcI—secondary spermatocyte; RS—round spermatid; ES—elongated spermatid; 2CS—two-cell stage; 4CS—four-cell stage. *Although the secondary spermatocyte carries 2n chromosomes, this is the first haploid cell in the male gametogenesis

to pregnant mice changed the methylation pattern of imprinted genes carried by spermatozoa up to two generations.[7,8] Their findings proved not only that the methylation pattern of sperm-carried DNA was not completely erased during spermatogenesis and fecundation, which can be inherited by the offspring, but also that environmental factors can change the localization and extension of methylation areas in sperm DNA. Soon other mechanisms of paternal transgenerational epigenetic inheritance were described. In 2014, Gapp *et al* demonstrated that the intracytoplasmic injection of RNA extracted from sperm of traumatized mice into fertilized eggs induced the same behavioural changes in the offspring as those observed in fathers.[9] One year later, another group published similar results, but using a rodent high-fat diet model.[10] Besides, these studies identified sncRNAs as responsible for coding this epigenetic inheritance. Although the amount of injected RNA was manyfold greater than the volume carried by a spermatozoon, these works clearly demonstrated that sncRNA content carried by sperm cells can also be responsible for the transgenerational-acquired phenotypes observed in the male lineage in mammals. Surprisingly, despite the small cytoplasmic volume of the spermatozoon, biparental inheritance of mitochondrial DNA was recently described in humans,[11] which further supports the role of paternal transmission of acquired traits.

3 | MALE EPIGENETIC SIGNATURES INFLUENCE *IN UTERO* DEVELOPMENT

Once several mechanisms underlying the transgenerational inheritance of acquired traits via the male gamete started to be unfolded, new questions arose. When do the epigenetic signatures exert their action? To what extent do they affect health outcomes in the offspring?

Following the study published in 2010,[8] Guerrero-Bosagna and colleagues have linked the changes in sperm DNA methylation pattern with sperm, testicular, prostate and kidney abnormalities.[12] According to the authors, the methylation pattern was inherited during gonadal sex determination, when male germline cells are remethylated according to the original methylation pattern carried by sperm DNA.[13] Histones can also be methylated causing chromatin condensation, hence inhibiting gene expression. This mechanism is particularly crucial in sperm DNA because retained histones are associated with critical genes for embryo development.[14] The demethylation of a histone subunit (H3K4me2) in mouse sperm, by action of a transgene, causes severe developmental impairment and decreases survival in the male offspring.[15] Additionally, although the offspring had not been exposed to the transgene, sperm histone demethylation

persisted for up to two generations and was identified even in the grand-grandsons of the mice exposed to the transgene. This study also elicits a crosstalk between diverse epigenetic mechanisms during embryonic development. At the two-cell stage, embryos of mice obtained from demethylated sperm had a reduced amount of RNAs, particularly sncRNAs.[15] This effect is diluted in subsequent generations, but considering that spermatozoa are transcriptionally silent, this means that chromatin condensation state may regulate gene expression of both coding and noncoding genes from the early embryo.

Considering the crosstalk between different epigenetic inheritance mechanisms, Donkin *et al* described a comprehensive profile of the epigenetic changes caused by obesity in human sperm.[16] They also compared the sperm epigenome of obese men before and after bariatric surgery. Additionally, the authors simulated the impact of those epigenetic markers in embryo development, using a bioinformatics analysis based on Gene Ontology (GO) terms. They found differences in the amount and distribution of DNA methylation in motile sperm between lean and obese men. Notably, protamine-bound regions contained more methylation changes than histone-bound DNA regions. Interestingly, most methylation changes occurred in genes related to embryonic development of the central nervous system and metabolism. Motile sperm from obese men presented higher amounts of sncRNAs, especially piRNAs related to chromatin condensation and food intake regulation.[16]

4 | NEO-LAMARCKISM, METABOLIC MEMORY AND HUMAN EVOLUTION

The advances in the knowledge of the mechanisms underlying the transgenerational transmission of epigenetic markers have proven that the inheritance of acquired traits is not stochastic or, in humans, it is not limited to socio-cultural factors. These findings have provided molecular evidence supporting to some extent the first theory on the evolution of species, postulated by Jean-Baptiste de Lamarck in the XIXth century. This rediscovering of Lamarck's evolution theory based on the transmission of acquired traits for generations has even been coined as neo-Lamarckism.[17] Indeed, recent data based on animal and human studies have demonstrated that epigenetic markers in spermatozoa dynamically respond to environmental factors, with long-lasting effects for several generations (Table 1).

These findings have a critical importance for public health, as humankind fights against the epidemic proportions of noncommunicable diseases, caused by lifestyle choices. However, studies involving lifestyle interventions such as dietary change and/or adiposity loss have provided inconclusive outcomes on the transmission of acquired traits to the offspring. Particularly, the development phase of the subject seems to be of critical importance to revert acquired traits. Recently, Crisóstomo *et al* demonstrated that mice fed with a high-fat diet from weaning to early adulthood, although they do not show long-lasting symptoms

TABLE 1 Selected studies eliciting a role of sperm epigenetic content in embryo development, and the associated developmental outcome

Study	Species	Ancestral exposure	Epigenetic mechanism	Outcome in the offspring
Stouder *et al* (2010)[7]	Mouse	Vinclozolin	Hypermethylation	Abnormal methylation pattern (somatic and germline cells)
Guerrero-Bosagna *et al* (2010, 2012)[a8,12]	Mouse	Vinclozolin	Abnormal methylation pattern	Abnormal methylation pattern (somatic and germline cells), sperm and testicular abnormalities
Gapp *et al* (2014)[9]	Mouse	Trauma/stress	Sperm-borne RNA (sncRNA)	Traumatic behaviour
Grandjean *et al* (2015)[10]	Mouse	High-fat diet	Sperm-borne RNA (mmu-miR-19)	Increased insulin resistance and body weight
Siklenka *et al* (2015)[15]	Mouse	Transgene (overexpression of hKDM1A)	Hypomethylation of retained histones (H3K4me2), reduced sncRNA	Impaired development, increased mortality (for 3 generations)
Donkin *et al* (2016)[16]	Human	Obesity, diabetes, high-fat diet, sudden weight loss	Abnormal methylation pattern (protamine-bound DNA regions)	Abnormal neuronal development, metabolic changes (theoretical)
Nätt *et al* (2019)[20]	Human	Short-term high-sugar diet	Overexpression of tsRNAs and mitochondrial sncRNAs in sperm	Abnormal tsRNA biogenesis, altered sperm mitochondria/cytosol compartmentalization (theoretical)

[a]Complementary studies.

of metabolic syndrome (contrary to mice fed with lifelong high-fat diet), have poorer sperm parameters and altered testicular lipidome and metabolome at adulthood, comparing with mice fed with standard chow.[18,19] These studies evidence that the SGP is critical to define the 'metabolic memory' of testicular metabolism, and consequently sperm quality later in life. The findings further corroborate the results of the earlier Överkalix study, which refers to the nutritional status during the SGP as a critical factor to influence health outcomes in the offspring.[5] Although this study does not report data on the sexual health of the cohorts, it is likely that the 'metabolic memory' is inherited by the offspring via spermatozoa and that it is coded by the epigenetic markers described in other studies.[12,15,16]

The epigenetic signatures carried by spermatozoa can also react to acute changes. Donkin *et al* have observed an extensive epigenetic remodelling of motile sperm of men as soon as 1 week after bariatric surgery, especially in the number of methylated genes.[16] Another group showed that nuclear tsRNAs, and mitochondria-derived rRNAs and tsRNAs are overexpressed in sperm of men exposed to an acute high-sugar diet.[20] It is likely that the changes observed in sperm epigenome, in response to nutritional status, affect embryo development, as demonstrated by earlier studies.[10,12,15]

The human species have freed itself from the laws of neo-Darwinism, but the views of neo-Lamarckism show that our success can be our most significant threat. Lifestyle is becoming a crucial element of transgenerational health, a driving force of human evolution. Therefore, it is essential that better health policies promoting healthy lifestyle choices are designed to prevent an escalade in the prevalence of morbidity attributed to noncommunicable diseases.

5 | CONCLUDING REMARKS

Modern Medicine has pushed for a more inclusive perspective of pregnancy, where mother and father equally share responsibilities towards the best health outcomes of the neonate. This effort has even been transcribed to the law in several countries, in different continents, where now fathers and mothers can enjoy parental leave to follow the baby for the first months of life. It is now time to push for more equality in the health follow-up of parents. The success of a pregnancy cannot be just attributed to the lifestyle, health status or simply physical 'inability' of the woman to bear a child. It is time to acknowledge the influence of the man, his lifestyle and environmental exposures, and his global health status at conception, in the development of progeny. During the XXth century, more educated and healthy women led to a dramatic decrease in child mortality and morbidity worldwide. It is time to invest also in more informed and healthy fathers. Healthier fathers will contribute to healthier children and healthier grandchildren.

However, we are just taking the first steps to understand the mechanisms of paternal transgenerational epigenetic inheritance. New research must clarify the signalling pathways involved in the epigenetic regulation in response to environmental stimuli. Single-cell omics can contribute to unravel specific epigenetic signatures responsible for different outcomes in response to the same stimuli. It is also largely unknown the relationship between acute or chronic environmental exposures and the extent and nature of the resulting epigenetic signatures. From a clinical perspective, it is crucial to integrate this knowledge in terms of 'health capital', that is to what extent epigenetic signatures are correlated with clinically significant phenotypes and how can we promote beneficial epigenetic changes in individuals.

KEYWORDS

epigenetics, metabolism, sperm, utero

ACKNOWLEDGEMENTS

This work was supported by the Portuguese Foundation for Science and Technology: L. Crisóstomo (SFRH/BD/128584/2017), MG Alves (IFCT2015 and PTDC/MEC-AND/28691/2017), UMIB (UID/Multi/00215/2019) and QOPNA (UID/QUI/00062/2019) co-funded by FEDER funds (POCI/COMPETE 2020); by the Portuguese Society of Diabetology: L. Crisóstomo and MG Alves ('Nuno Castel-Branco' research grant and Group of Fundamental and Translational Research).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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How to cite this article: Crisóstomo L, Oliveira PF, Alves MG. Sperm, metabolic memory and echoes from Lamarck. *Eur J Clin Invest*. 2021;00:e13492. https://doi.org/10.1111/eci.13492