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Molecular basis of spermatogenesis and sperm quality

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ABSTRACT

Spermatozoan quality can be evaluated in different ways, here we focus on the analysis of DNA, RNA and epigenetic status of germ cells. These characterizations also can be the bases for explaining sperm quality at other levels, so we will see how some of these molecules could affect other sperm quality markers. Moreover, we consider the possibility of using some of these molecules as predictors of sperm quality in terms of the ability to produce healthy offspring. The relevant effect of different types of RNA molecules in germ line specification and spermatogenesis and the importance of germ cell DNA integrity and a proper epigenetic pattern will be also discussed. Although most studies at this level have been performed in mammals, some information is available for fish; these recent discoveries in fish models are included. We provide a general overview on how these molecules could have a deep influence in the final sperm quality.

1. Functions of non-coding RNAs in the control of germline development: miRNAs, piRNAs and lncRNAs

RNAs are crucial from very early stages in germline development. Primordial germ cells (PGCs) (the precursors of gametes) acquire a specific gene expression program in which non-coding RNAs and ribonucleoproteins play an important role. Some ribonucleoproteins (RBPs) such as DND1 are from very early stages mainly expressed in germ cells having an essential role in germline development. They are such important proteins that if in zebrafish embryos their translation is blocked by using morpholinos, PGCs do not differentiate and adults which are derived from these embryos never develop functional gonads (Riesco et al., 2014). Micro RNA (miRNAs) are non-coding RNA molecules with the capacity to regulate other gene expression by different processes and it is known that differential susceptibility to micro RNAs contributes to tissue-specific gene expression. As an example, miR-430 targets the 3́UTRs of mRNAs from germline genes (Tani et al., 2010). In order to prevent degradation of mRNAs that are crucial for germline development, DND1 binds uridine rich regions in the 3′UTR, either sequestering mRNAs or physically displacing miRNA-Induced Silencing Complex (miRISC) to alleviate micro RNA mediated suppression (van Kouwenhove et al., 2011) and therefore, preventing mRNA from degradation.

If PGC specification and migration to the genital ridge is successful, and a functional gonad develops, spermatogenesis will take place. RNAs are also crucial in spermatogenesis. It is well known that male germ-cell differentiation is tightly controlled at transcriptional and post-transcriptional level. Transcriptional and

Abbreviations: BPA, bisphenol A; CB, deoxyribonucleic acid; Ct, threshold cycle; DCP1a, decapping mRNA 1A; *Dmr*, doublesex and mab-3 related transcription factor; DNA, Deoxyribonucleic acid; DND, dead end protein; ICSI, intracytoplasmatic sperm injection; Insrβ, insulin receptor beta; KIF17b, kinesin family member 17 b; lncRNA, long non-coding RNA; miR-430, micro RNA 430; miRISC, miRNA-mediated silencing complex; miRNA, micro RNA; mRNA, messenger RNA; MVH, mouse vasa homolog; PGC, primordial germ cell; piRNA, piwi-interacting RNA; piwi, P-element induced wimpy testis; qPCR, quantitative polymerase chain reaction; RBP, ribonucleoproteins; RNA, ribonucleic acid; siRNA, small interfering RNA; Spga-lncRNA 2, spermatogonia-specificlncRNA 2; Spga-lncRNA1, spermatogonia-specificlncRNA 1; Tsx, testis specific X-linked gene; UTR, untranslated region; zili, zebrafish piwi like RNA-mediated gene silencing 2 homolog; ziwi, zebrafish piwi homolog.

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post-transcriptional regulation are crucial processes that allow the cells to have a rigorous control in time and space over the genetic information displayed. Post-transcriptional regulation is particularly important during the late steps of spermatogenesis when the compacting sperm nucleus becomes transcriptionally inhibited (Yadav and Kotaja, 2014). In post-transcriptional processes, target mRNAs are controlled by RNA binding proteins and by non-coding RNAs (Yadav and Kotaja, 2014).

Male germ cells express several classes of small RNAs including Dicer-dependent micro-RNAs (miRNAs) and endogenous small interfering RNAs (siRNAs) as well as Dicer independent piwi interacting RNAs (piRNAs) (Meikar et al., 2011). Both miRNAs and siRNAs control mRNA translation and degradation either by triggering endonuclease cleavage, promoting translation repression, or accelerating mRNA decapping (Valencia-Sanchez et al., 2006; Yadav and Kotaja, 2014). The differences between them are in their biogenesis. First type is produced from stem loop transcripts and second type is produced from long double stranded RNA precursors (Yadav and Kotaja, 2014). Both types are Dicer dependent for the cytoplasm-endonuclease-processing, but miRNAs are also early processed in the nucleus by Drosha. In mammals it is well known that different miRNAs are crucial for different steps of spermatogenesis. Some of them are relevant for the maintenance of undifferentiated state of spermatogonia, others in the induction of differentiation, and some others will have an important role in early embryo development (Fig. 1) (Kotaja, 2014; Luo et al., 2015; Wang and Xu, 2015). Although miRNAs in fish have not been as studied as those in mammals, deep sequencing profiling in some fish species (*Takifugu rubripes*) have demonstrated that most miRNA sequences are conserved, which indicates that the basic functions of vertebrate miRNAs share a common evolution. Moreover, some miRNAs families are abundant in the gonads, but are expressed only at low levels in somatic tissue; this finding suggests miRNA has a specific function in germ cells (Wongwarangkana et al., 2015). Several miRNAs were reported to be particularly abundant in rainbow trout testis (Farlora et al., 2015) and a recent study revealed that dre-miR-202-5p is common in zebrafish spermatozoa and testis, suggesting that this miRNA might be related to spermatogenesis and spermatozoa functioning (Jia et al., 2015).

Germ cells also possess piRNAs. PiRNAs are single stranded RNAs which are present in high numbers in the male germ line. Although PiRNAs sequences are not conserved between species PiRNA clusters seems to be conserved. The PIWI pathway has an important effect on testis differentiation and development and it is also important in genome defense against transposable elements which contribute to genome integrity maintenance (Bao and Yan, 2012; Siomi et al., 2011). Moreover, piRNAs are also considered as potential mediators of epigenetic transgenerational inheritance. The zebrafish genome encodes two Piwi homologs, Ziwi and Zili (Houwing et al., 2007). As has been demonstrated in mouse Piwi mutants, *Ziwi* mutants display a progressive decline in the germ cells due to apoptosis (Houwing et al., 2007). Moreover, as a result of PGC loss, Ziwi mutants are phenotypically males, since PGCs are required during embryogenesis for female development (Dranow et al., 2013).

Not only are small non-coding RNAs crucial for germline development, Long non-coding RNAs (lncRNAs) are also relevant. Interestingly, this group of RNAs, that are transcribed by RNA polymerase II, or formed by processing other transcripts, do not have a unique association with a specific group of proteins for their processing and function, contrary to what was observed for small noncoding RNAs which depends on argonaut (Yadav and Kotaja, 2014) and PIWI proteins (Chuma and Nakano, 2013). These RNAs could act at different levels having a huge variety of regulatory roles: they could inhibit miRNA function, avoid translation, influence chromatin remodeling, promote DNA methylation or even act as a precursors of short RNA (Kung et al., 2013). It is known in mammals, that some lncRNAs have specific roles in male germ cell development. As an example, Testis-specific X-linked (*Tsx*) is relevant for progression of meiosis, *Dmrt1*-related gene (*Dmr*) is probably involved in the switching between mitosis and meiosis of the germ cell development and *Spga-lncRNA1* and *2*, may be important in maintaining spermatogonia stemness. LncRNAs expressed in different germ cells have been identified in mice: 50 expressed in type A spermatogonia, 35 in pachytene spermatocyte, 24 in round spermatids (Lee et al., 2012).

But do the germ cells have a specific RNA processing centre in which these regulator pathways could converge? A centre for RNA storage and metabolism? This is the Chromatoid Body (CB),

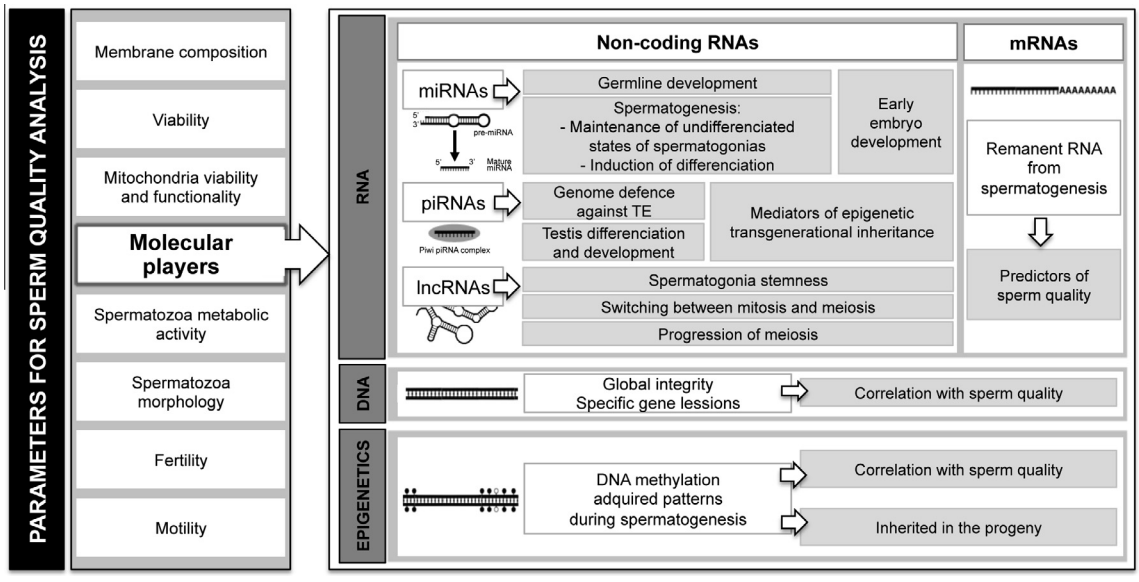

Fig. 1. Molecular players used as parameters for sperm quality analysis and their roles on male reproductive key process. RNA: non-coding RNAs (miRNAs, piRNAs and lncRNAs) and mRNAs; DNA and epigenetic status.

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