Sperm and seminal plasma microRNAs in the context of bull sperm fertility

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Abstract

Spermatogenesis consists of mitotic proliferation of spermatogonia, meiosis of spermatocytes, haploid differentiation of spermatids, and genesis of male gametes. Male germ cells have a complex transcriptome. Phase-specific gene expression patterns are seen by the active transcription of the genome of male germ cells, however new RNAs are not transcribed in haploid sperm. The RNAs originated from the diploid germ stem cells are carried to the oocyte upon fertilization. Sperm have protein-coding messenger RNAs, and many noncoding RNAs, including microRNAs (miRNAs). The miRNAs regulate gene expression, mainly by repressing post-transcription or by inhibiting translation of their target messenger RNAs. Clinical studies have exploited the well-defined profiles of miRNAs in sperm and seminal plasma, and human sperm or seminal plasma miRNAs have been proposed as potential biomarkers for male factor infertility. We have explored the expression of miRNAs in bull sperm or seminal plasma and correlated with their field fertility. Remarkably, a few studies explored miRNome profiling in bull sperm and seminal plasma using deep sequencing and mapping technologies. In future studies, whole-miRNome profiling using highly sensitive and specific deep sequencing and mapping technologies should be used to examine bull sperm and seminal plasma. Once novel and known miRNAs are screened by deep sequencing technologies, functionality should be validated using realtime PCR technology. Most of all, standardization of the nomenclature and abundancy specific to cattle is needed to include miRNome profile in bull fertility testing.

Contents

MicroRNAs (miRNAs), a recently discovered class of non-coding RNAs, function as epigenetic regulators of gene expression, either by degrading target mRNA or inhibiting its translation. Consequently, miRNAs have critical roles in regulating development and function. In addition, expression of miRNAs in tissues and biofluids are perturbed in developmental and functional aberrations, making them as promising molecular biomarkers for various cell types, tissues and biofluids.

Sperm have thousands of RNAs, including messenger RNA (mRNA), micro RNA (miRNA), interference RNA (RNAi) and antisense RNA (asRNA). Since sperm chromatin is condensed and compacted until fertilization, these sperm RNAs are not transcribed from sperm nuclear DNA, but they originate from diploid cells (spermatogonia and primary spermatocytes) and subsequently contribute to fertilization and embryo development.[1-3] Sperm-borne miRNA-34c has been identified to be required for the first cell division in mouse zygote.[4] The expression levels of miR-21, miR-34c, miR-140 and miR-375 were positively correlated with number of blastomeres and embryo quality.[5] Based on expression levels of the miR-34 family in bovine sperm, oocytes and embryos, these miRNAs were implicated in transfer of epigenetic messages from gametes to offspring.[6] In addition, MiR-30d-5p, miR-320b, miR-10b-3p, miR-1291, and miR-720 were predominant in follicular fluid in women undergoing *in vitro* fertilization, and expression levels of miR-202-5p, miR-206, miR-16-1-3p, and miR-1244 in follicular fluid varied significantly between follicles containing a fertile versus non-fertile oocyte.[7] Epigenetic signatures including miRNAs in germ cells are vulnerable to the alterations in parental environment, and these epigenetic signatures can influence the embryo development and can mediate transgenerational programming.[8,9] RNA molecules even in transcriptionally inert mature sperm are of primary interest, as they play important roles in transgenerational programing and these RNA population may be altered by environmental change, even via intercellular communication of epididymosomes.

In dairy and beef cattle production, bull fertility is an important economic trait, although cow fertility is more often considered in genetic selection. Male and female fertility have low heritability values; however, genetic and epigenetic factors from both gametes influence fertilization and embryo development. With increased use of artificial insemination and other assisted reproductive technologies in cattle breeding, semen quality and sire fertility are scrutinized continuously. Decision to use or discard the

semen is often based on semen quality or field fertility. No single sperm bioassay is presently available to accurately predict bull fertility, however, many sperm assays have shown to be highly correlated with *in* vivo fertility. Although various powerful tests including computer-assisted sperm analysis and various flow cytometric analyses have been developed, their accuracy and repeatability are still uncertain. Sperm RNA profiling is a potential tool to assess sperm fertility, as they regulate sperm functions, including motility in the female reproductive tract, capacitation, acrosome reaction, fertilization and post-fertilization events. Therefore, sperm-borne miRNAs could serve as epigenetic regulators of genes required for fertilization and post-fertilization events. These sperm-specific miRNAs can alter gene expression in a heritable manner, without transgenerational alterations of DNA sequences, in addition to histone modification, DNA methylation and acetylation.[10] Bull sperm have many miRNAs; expression level varied between bulls differing in non-return rates, implying they may be a marker of bull fertility.[11] Several miRNAs were differentially expressed between high- and low-fertility Holstein bulls; targeted gene expression analyses confirmed involvement of these differentially expressed miRNAs in spermatogenesis and early embryonic development.[12] In frozen-thawed sperm, 178 miRNAs were investigated, of which seven (mir-502-5p, mir-1249, mir-320a, mir-34c-3p, mir-19b-3p, mir-27a-5p and mir-148b-3p) were differentially expressed between high- and moderate-fertility Holstein bulls.[13]

Seminal plasma miRNAs were stable, with 692 miRNAs identified in human seminal plasma by Solexa sequencing. In addition, 19 were altered in count between azoospermia and asthenozoospermia patients and a control group.[14] Two selected seminal plasma miRNAs, miR-19b and let-7a, differed in expression level between idiopathic infertile males and fertile controls.[15] Thirty-seven miRNAs were differentially expressed in seminal plasma of cancer patients compared to control, with upregulated miR-142 and downregulated miR-34b validated using RT-qPCR.[16] Five miRNAs (hsa-miR-34b, hsa-miR-34b, hsa-miR-34c-5p, hsa-miR-429, and hsa-miR-122) were variously expressed in seminal plasma of infertile patients and proposed as seminal plasma markers for infertile humans. We profiled 84 highly prioritized bovine miRNAs in seminal plasma of high- and low-fertility bulls and identified 56 miRNAs differentially expressed (Table; Kasimanickam & Kasimanickam, unpublished data). Furthermore, in boar semen, there was an association of normalized threshold cycle values of sperm and seminal plasma microRNAs (Figure 1; Kasimanickam & Kasimanickam, unpublished data).

Summary

In summary, numerous microRNAs are present in sperm in humans and animals. These miRNAs may originate from germ stem cells, are relatively inert in sperm, but carry messages to oocytes and can influence fertilization and postfertilization events without altering DNA sequences. Additionally, miRNAs are abundant in seminal plasma. The miRNAs in seminal plasma are stable and easily quantified. Seminal plasma miRNAs may originate from sperm or they may shuttle between sperm and seminal plasma to facilitate cell-to-cell communication. Sperm and seminal plasma miRNAs are differentially detected during developmental progression, whereas expression levels are altered in pathologic conditions. In most situations, expression levels of miRNAs and their target genes are indirectly proportional. High-throughput sequencing and mapping technology (Figure 2) continue to identify known and novel miRNAs in sperm and seminal plasma, although these technologies are mostly adopted for humans. Prediction and pathway analysis identify target genes and clarify epigenetic function. Notably, a few studies explored miRNome profiling in bull sperm and seminal plasma using deep sequencing and mapping technologies. In future studies, whole-miRNome profiling using highly sensitive and specific deep sequencing and mapping technologies should be used to examine bull sperm and seminal plasma. Once novel and known miRNAs are screened by deep sequencing technologies, functionality should be validated using realtime PCR technology. Most of all, standardization of the nomenclature and abundancy specific to cattle is needed to include miRNome profile in bull fertility testing. To further explore their role in spermatogenesis, temporal profiling of miRNAs in various cell types, spermatogonia, primary spermatocytes, secondary spermatocytes, and spermatids are essential.

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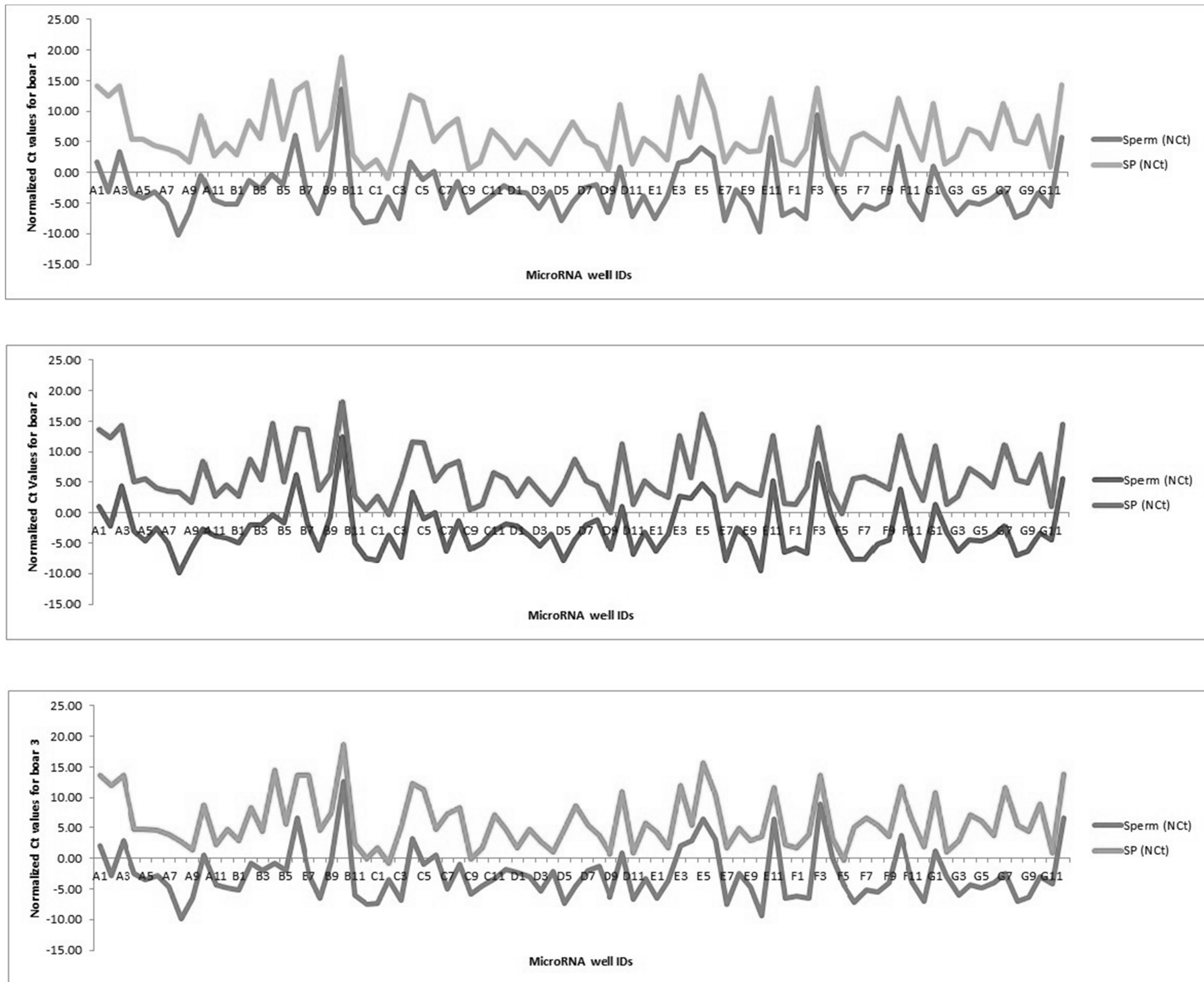
Table: Relative expression of seminal plasma miRNAs between high- and low-fertility Holstein bulls.

Layout	01	02	03	04	-05	06	07	08	09	10	11	12
A	bta-let-7f -4.05	bta-miR-101 -10.99	bta-miR-103 -1.20	bta-miR-125a -2.38	bta-miR-125b -2.23	bta-miR-126-3p 1.12	bta-miR-128 -5.75	bta-miR-145 5.07	bta-miR-148a -5.65	bta-miR-151-3p 1.09	bta-miR-151-5p 5.91	bta-miR-16b -32.60
B	bta-miR-181a 1.41	bta-miR-18 -1.46	bta-miR-18b 2.02	bta-miR-199a-5p 20.11	bta-miR-205 -1.81	bta-miR-20a -1.09	bta-miR-21-5p 3.84	bta-miR-221 1.66	bta-miR-222 1.06	bta-miR-26a -3.71	bta-miR-26b -3.21	bta-miR-27a-3p -1.76
C	bta-miR-27b -1.91	bta-miR-29a -9.26	bta-miR-30b-5p -1.50	bta-miR-30d 2.35	bta-miR-31 8.37	bta-miR-320a 5.09	bta-miR-34b -8.60	bta-miR-484 7.70	bta-miR-499 1.14	bta-miR-99a-5p -7.97	bta-miR-5p -1.55	bta-let-7d -1.37
D	bta-let-7g -1.87	bta-let-7i 1.08	bta-miR-17-5p -1.01	bta-miR-107 7.57	bta-miR-10a -4.41	bta-miR-10b -3.66	bta-miR-122 1.26	bta-miR-124b 2.41	bta-miR-127 3.32	bta-miR-132 2.65	bta-miR-138 6.64	bta-miR-139 6.55
E	bta-miR-140 3.33	bta-miR-142-3p 3.15	bta-miR-142-5p 13.45	bta-miR-148b -1.07	bta-miR-150 7.14	bta-miR-15b -6.51	bta-miR-17-3p 4.01	bta-miR-17-5p 8.61	bta-miR-181b 4.80	bta-miR-181c 1.46	bta-miR-186 -8.50	bta-miR-191 -5.34
F	bta-miR-192 1.66	bta-miR-193a-3p 12.83	bta-miR-193a-5p 6.93	bta-miR-1991-3p 9.03	bta-miR-199b 6.80	bta-miR-200a -11.03	bta-miR-200b -2.59	bta-miR-200c -1.57	bta-miR-20b 17.78	bta-miR-210 4.40	bta-miR-21.3p 13.48	bta-miR-214 23.68
G	bta-miR-215 7.20	bta-miR-218 2.52	bta-miR-22.5p 1.16	bta-miR-23a -1.39	bta-miR-23b-3p -1.48	bta-miR-24-3p 2.35	bta-miR-25 -2.15	bta-miR-29b -2.66	bta-miR-29c -14.58	bta-miR-30a-5p 1.50	bta-miR-30c -1.97	bta-miR-30e-5p -4.09

Positive numbers indicate upregulated miRNAs and negative numbers indicate down regulated miRNAs in high fertile bulls (SCR +7; n=3) compare to low fertile bulls and high (SCR -4; n=3)

Figure 1. Normalized threshold cycle values for sperm and seminal plasma miRNAs for three high-fertile Landrace boars.



Within a boar, normalized cycle values had similar trends for sperm and seminal plasma miRNAs

Figure 2. Deep sequencing and mapping analyses of RNA from sperm or seminal plasma.