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RESEARCH PAPER

New Approaches to Predict the Sperm Quality by the Spermatozoal miRNA Content in Fish: A Review

Filiz Kutluyer Kocabaş[1], Mehmet Kocabaş[2,*]

[1]Munzur University, Fisheries Faculty, Tunceli, Türkiye.
[2]Karadeniz Technical University Faculty of Forestry, Department of Wildlife Ecology and Management, 61080, Trabzon, Türkiye.

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Corresponding Author
E-mail: mkocabas@hotmail.com

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Abstract

The survival of the progeny and fertilization success depend on high-quality mature spermatozoa containing DNA and coding and non-coding RNAs besides the female gamete quality. Sperm cells and gonads in fish contain different RNA classes. It is possible that the development of fish could be affected by miRNA and/or other non-coding RNAs being abundant in Teleost fish and having important functions. Studies about the presence of miRNA in some fish species (e.g. *Danio rerio*, *Salmo salar*, *Carassius auratus, Cyprinus carpio*) showed that sperm motility is regulated by miRNA. Nevertheless, limited knowledge and several hypotheses have been presented about classes and content of RNAs in fish spermatozoa. More research about the effects of miRNA on fish reproduction and sperm quality are needed for population, aquatic ecosystems, and broodstock management, and the evaluation of molecular sperm quality parameters with miRNA could be used together with traditional techniques.

Introduction

The reproduction in fish is of vital importance to ensure the existence of populations (Dreanno et al., 1999; Linhart et al., 2000; Rurangwa et al., 2001; Bozkurt and Seçer 2006; Cuadrado et al., 2014; Xiong et al., 2018). Fish is not considered to have adapted to the environment if they do not have the ability to reproduce. The genetic capacities of species are used for assessing the reproductive success related to ecological conditions. In this context, each species has its own reproductive strategy. Therefore, reproduction is the set of strategies and tactics that has been developed to ensure the continuity of the species in the evolutionary process (Muchlisin, 2014). Within this integrity, there are many environmental adaptations such as age of sexual maturity, choice of breeding area, period of reproduction, amount of gametes produced, and mode of reproduction. For successful fisheries management, it is essential to assess the species' reproductive biology in terms of fish farming (Kutluyer, 2018).

The quality of gametes plays a primary role in aquaculture production. Many biotic and abiotic factors affect gamete quality of fish in natural or cultured conditions (Islam and Akhter, 2011; Kutluyer et al., 2014). Biological gamete quality can be described as the ability of a sperm to fertilize or an egg to be fertilized and turn into a normal embryo. In addition, the quality of gametes could be explained by special biotechnical differences in the applications depending on the use of gametes (e.g. androgenesis, nuclear transfer) (Bobe and Labbé, 2010).

The term "gene" has been defined for many years as parts of the genome that are translated into proteins and encode miRNAs (Ying et al., 2008). However, in recent years, genome sequencing studies at large-scale have demonstrated that humans and mice share a similar number of protein-coding genes contrary to expectations, and complex organisms will have a greater number of genes. In fact, the number of protein-coding genes in most advanced living things is in some single-celled organisms (Dikme et al., 2022). In gene regulation, miRNA is essential as the critical element (Herkenhoff et al., 2018). In the reproductive processes, the miR-200 family have an important role in mouse (Hasuwa et al., 2013). In vertebrates, *amh*, *dmrt1, wt1sox9,* and *sf1* have been identified as master genes for the process of spermatogenesis and male sex differentiation (Cutting et al., 2013; Mei and Gui, 2015; Xiong et al., 2018) while *wt1, amh, galectin-1, crisp1, dmrt1*, *sf1, gsk3a,* and *srd5a* were correlated with sperm capacity and spermatogenesis (Xiong et al., 2018). Hence, sperm activity, fertility, and hatching rates are under the influence of expression of these genes. In this context, the determination and importance of micro RNA (miRNA) content of fish sperm cells were presented in the review.

Structural Sperm Features of Fish

The male germ cell of fish is called the spermatogonia and is composed of three main parts: a head, a middle piece, and a tail (Kayalı et al., 1992; Lahnsteiner and Patzner, 2008) (Figure 1).

Head is oval and pear-shaped. In dyed preparations, the back of the head is painted very darkly with core dyes due to containing highly condensed DNA. This part of the head plays an important role in fertilization, carrying enzymes released during maturation in the spermatozoon epithelium (Lahnsteiner and Patzner, 2008).

The midpiece consists of two parts, the neck and the connecting part. The neck is very short and consists of a head plate (*Moduli anteriores*) and an intermediate-mass (*Massa intermedia*). Since the neck acts as a mobile, the head part gains the ability to move against the rest of the spermatozoa. In the combining part; transverse disc (*Discus transversalis*), end ring, axial thread, spiral thread, and cytoplasmic sheath. The transverse disc is just a motor. The stability of this plate is essential for the spermatozoa to move. The last ring (bottom ring or occlusive ring) is a plate of the posterior centriole. The axial thread consists of a fibril starting from the transverse disc and continuing on the tail. The spiral thread is an 8-9 twisted thread made of mitocontriles that wraps the thin plasmatic sheath around the axis thread. The cytoplasmic sheath is the outermost thin membrane (Kayalı et al., 1992; Lahnsteiner and Patzner, 2008, Köprücü, 2018).

The tail is the thinnest and longest part of the spermatozoa. It consists of a long main part (*Pars principalis*) and a short last part (*Pars terminalis*). The axial filament runs along the entire tail. The tail allows the sperm to move forward with snake-like movements. When the sperm is examined as a cell, the head appears to fit into the core (DNA) and all other parts of the cell body. Because it contains genes and plays the main role. Like the ship's propeller, the tail is only a part that provides movement. The headless spermatozoa can move if its transverse plate is intact. As it can be understood from here, the spermatozoon has high motility, sometimes its fertilising ability can be low.

Micro RNA

As a post-transcriptional regulator MicroRNA (miRNA) was discovered in 1993 for gene expression in *Caenorhabditis elegans* simultaneously in two independent studies (Lee et al., 1993; Wightman et al., 1993). It was observed that the miRNA discovered as Lin-4 decreased the expression of the protein product, and the target gene was not found in DNA sequence analysis (Lee et al., 1993). Although protein-coding gene expression occurs in a small portion (0.5-2%) of the genome, miRNAs constitute only 1-2% of mammalian, fly and worm genes. In addition, hundreds of target genes are controlled by each miRNA (Bartel, 2009).

MicroRNAs (miRNA) are single-stranded and short RNAs (22-24 nucleotides in length). It has been found that genes encoding miRNAs are conserved among many different species (Bartel, 2009). These properties highlight the importance of the roles of these small molecules in physiological processes. It has been determined that there are over 1800 miRNAs in humans (The miRBase Sequence Database, 2016), and it is predicted that these miRNAs regulate human genes (approximately 30-60%) (Lewis et al., 2005; Friedman et al., 2009). miRNAs function is at the post-transcriptional level by binding to the 3'UTR region of mRNA (Bartel, 2009), at the transcriptional level by binding to the start sites of genes (Place et al., 2008), or by acting in epigenetic processes (Rodgers et al., 2013). When these regulatory molecules bind to the 3'UTR region of the mRNA of the target gene, they cause suppression or inhibition of gene expression at the post-transcriptional level (Bartel, 2009). A single miRNA can have multiple target genes and a single gene can be regulated by more than one miRNA simultaneously (Sood et al., 2006). Mature miRNAs are important in the control of essential physiological processes (e.g. cell differentiation, cell cycle) (Le Bot, 2012), growth, and apoptosis (Cheng et al., 2005). Furthermore, Mineno et al. (2006) have demonstrated that they are involved in processes linked to gamete development during embryonic development, and Eisenberg et al. (2015) have found associations with female reproductive activities such as ovulation and corpus luteum development, while Björk et al. (2010) and Maatouk et al. (2008) have reported their involvement in male reproductive functions such as spermatogenesis and spermiogenesis. On the other

hand, there is information that a mutation that may occur in miRNA sequences, a defect in their biogenesis (Khazaie and Esfahani, 2014), or polymorphisms in gene sequences may lead to infertility (Zhang et al., 2011). For this reason, it is thought that examining spermatozoal and seminal miRNAs in the investigation of male factor-related infertility will shed light on the treatment and diagnosis process (Barbu et al., 2021).

Biogenesis of miRNAs

In the nucleus, the transcription mediated by RNA polymerase II initiates the miRNA biogenesis, and pri-miRNA (a long miRNA) is formed with the mature miRNA sequence in the hairpin structure (Winter et al., 2009) (Figure 2). The microprocessor comprised of RNAase III enzyme (Drosha) and its cofactor DGCR8 (DiGeorge critical syndrome region 8) (Pasha), resulting in the formation of pre-miRNA (precursor miRNA) with a length of 60-70 nucleotides, cut the hairpin structure. Exportin-5 transport (XPO5) the precursor hairpin from the nucleus to the cytoplasm and is cut into a 21-24 nucleotide duplex miRNA by another RNAase III enzyme, Dicer. The strand to be cut into the mature sequence is loaded into the Argonaute (RISC), forming the miRNA-induced silencing complex. With missing base pairing, miRNA induces RISC and causes mRNA destabilization or translational suppression.

Biomaterials Containing miRNAs

Differentially expressed miRNAs between tissues are found in the cell, extracellular, circulation, and body fluids. Tissues, cells, and fluids have their own miRNAs (Guo-Hua, 2014; Dikme et al., 2022). miRNAs are found in biological fluids such as cerebrospinal fluid (CSF), pleural effusion, urine, eye fluid, saliva milk, bile, blood, and plasma (Javidi, 2014; Dikme et al., 2022).

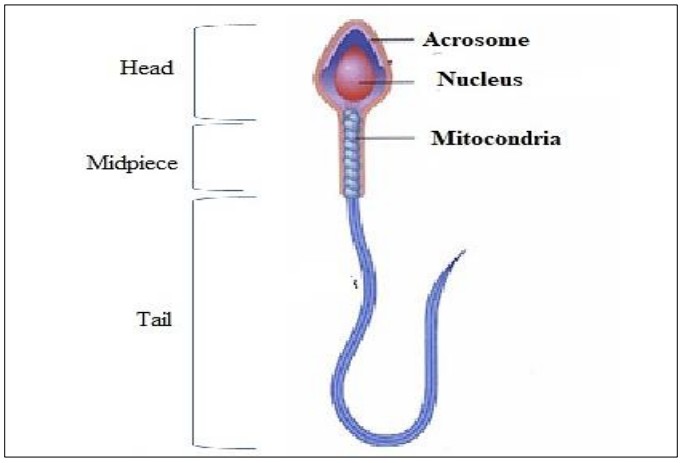

Figure 1. Structural sperm features of fish adapted from Kayalı et al. (1992)

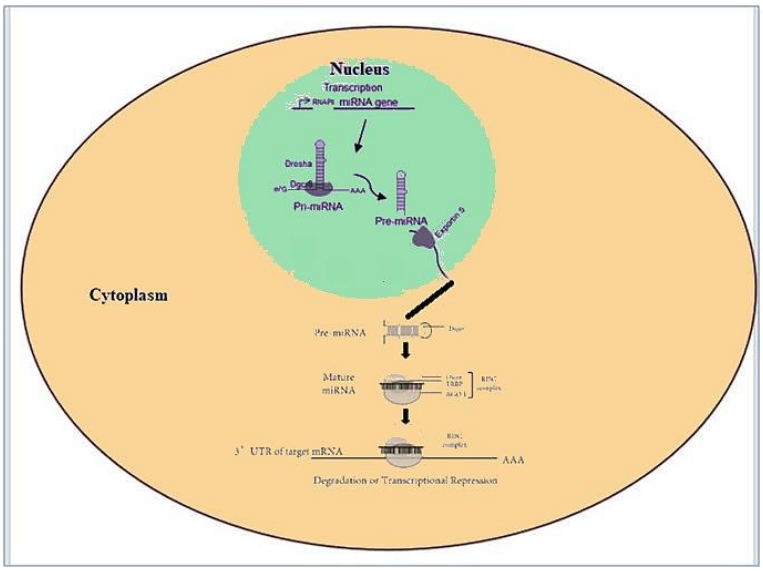

Figure 2. A schematic of miRNA biogenesis adapted from Hajarnis et al. (2015) and Pisarello et al. (2015) (Drosha: RNAase III enzyme, DiGeorge critical syndrome region 8, pre-miRNA: precursor miRNA, XPO5: Exportin-5 transport

The Methods Used in the Assessment of miRNA Content

The regulatory mechanisms in miRNAs are assessed by integrating mRNA and miRNA expression data associated with next-generation sequencing or microarray analysis. On the other hand, it is difficult to obtain results using this approach for translationally repressed miRNA targets (mRNAs). Therefore, using mRNA sequences to characterize miRNA-target regulation may result in missing real targets, so this approach may be inaccurate (Dikme et al., 2022).

RNA isolation techniques (the column and chemical-based) have been used effectively for the isolation of mRNA, and these methods have restrictions regarding their use for miRNA (Moldovan, 2014). There are two commonly used approaches for the evaluation of miRNA levels. These approaches are methods based on qPCR (quantitative PCR) and sequencing (Mestdagh, 2014).

Define miRNA targets allows the definition of targets regulated at the translation level and uses Ago2 immunoprecipitation. To enhance mRNAs integrated into RISC, Ago2 immunoprecipitation is used and thus targeted by a miRNA (Dikme et al., 2022).

As the critical steps, the evaluation and transparency of miRNA data have been important in recent years. Other strategies (a few stable reference genes or global mean normalization) can be used to solve this problem (Mestdagh, 2014; Zhao et al., 2017). In order to enhance the transparency of the evaluation, detailed information about the name and sequence of miRNA should be given in the publications (Van Peer, 2014). Software such as miR-tracker can be used for this (Dikme et al., 2022).

The Gonadal miRNA Content in Teleost

The multiple biological processes such as apoptosis, immunity, differentiation and proliferation of cells, energy metabolism, gametogenesis, embryonic development and, metamorphosis are regulated by miRNAs (Figure 3) (Brennecke et al., 2003; Yeh et al., 2014; Nixon et al., 2015; Vienberg et al., 2017; Wang and Zhu, 2017; Conine et al., 2018; Qi et al., 2018; Li et al., 2020; Alvi et al., 2021). Micro RNAs (miRNAs) are found in the sperms of fish in vertebrates. These non-coding RNAs are about 22 nucleotides long, and the regulation of biological processes, including spermatogenesis, occurs by translation suppression resulting from binding to the 3′ UTR of mRNA (Yadav and Kotaja, 2014; Bizuayehu and Babiak, 2020). Micro RNAs (miRNAs) families have been reported in the gonads of different fish species (Table 1).

The role of miRNA on Fish Spermatogenesis

The role of miRNA in fish spermatogenesis is illustrated in Figure 4. In testis, the proliferation and maintenance of Leydig and Sertoli cells as germ cell-supporting somatic cells are provided by miRNAs (Bizuayehu and Babiak, 2014). In particular, the Sertoli

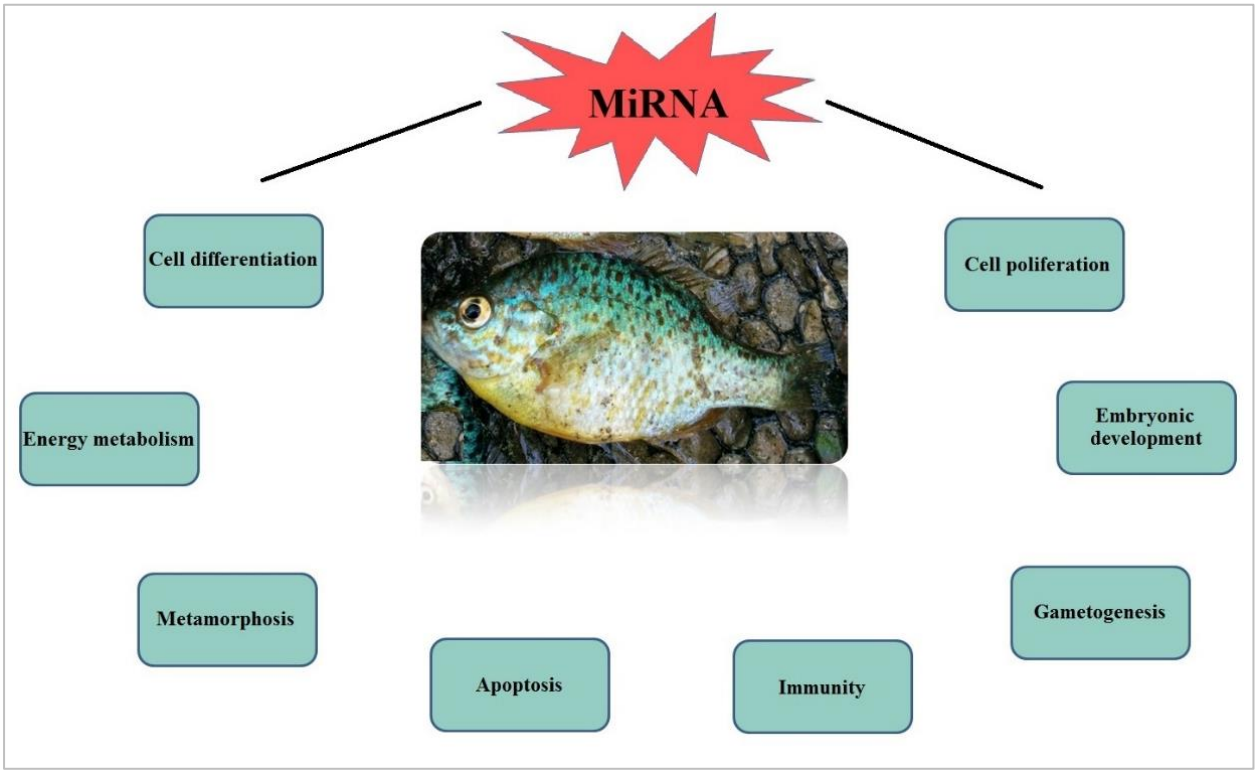

Figure 3. Summarize the potential roles of miRNAs in the multiple biological processes of fish

cell number of sexually mature fish is important in sperm production as a limiting factor (Schulz et al. 2010). During spermatogenesis, the role of miR-872, miR-24, and miR-125a-3p in translational control has been reported by Papaioannou et al. (2010) with the Sertoli cell-specific Dicer conditional knockout mouse model. Primordial germ cells (PGCs) carry the genetic information. During embryogenesis, gametogenesis in fish starts with asymmetric mitotic divisions of PGCs (Bizuayehu and Babiak, 2014). The migration of PGCs to the genital ridges is realized, and then PGCs are transformed into oogonia or spermatogonia during sex differentiation after the formation of gonocytes (Lubzens et al. 2010; Richardson, et al. 2010; Schulz et al. 2010). Köprunner et al. (2001) and Mickoleit et al. (2011) reported the importance and necessity of *tdrd7, Nanos, and hub* for the maintenance, survival, and migration of PGCs. In PGCs, 3'-UTR-binding sites are protected by Dead end (Dnd) (RNA-binding protein) from miR-430-mediated repression (Kedde et al. 2007). Qiu et al. (2018) reported the presence of miR-202-5p in medaka testis during all stages of spermatogenesis and decreased until the development of early spermatids. In addition, they demonstrated that the spermatogonia and spermatocytes contain the *Opiwi* abundantly while the presence of the *Opiwi* in spermatids and sperm was minimal level in medaka.

The Importance of Assessing the Spermatozoal miRNA Content in Fish

In reproductive events, fertility and hatching rates are positively correlated with sperm quality (Dreanno et al., 1999; Linhart et al., 2000; Rurangwa et al., 2001; Bozkurt and Seçer 2006; Cuadrado et al., 2014; Xiong et al., 2018). In studies conducted to understand and improve sperm physiology and quality, determining the number and motility of sperm cells in the sperm fluid is the most widely used parameter to define sperm quality (Bromage and Roberts, 1995). However, all physical

Table 1. The gonadal microRNA families in Teleost fish species (miRNA:microRNA)

Species	MiRNA families		Researchers
	Ovary	Testes	
Hippoglossus hippoglossus	miR-145, miR-143, miR-199-3p, miR-199, miR-202-3p, miR-100, miR-9-3p, miR-125b, miR-19b- 3p, miR-19b	let-7a, miR-143, miR-145, miR-202-3p	Bizuayehu et al. (2012)
Pelteobagrus fulvidraco	miR-21-5p, miR-21-3p, and miR-462-5p	miR-9-3p, miR-103b-3p, and miR-7b	Jing et al. (2014), Wang et al. (2018)
Paralichthys olivaceus	miR-143, miR-181a, miR-10c, let-7c, miR-21, let-7a, let-7f, let- 7 g, miR-100-5p, miR-92a	pol-miR-9-5p, pol-miR-182-5p, pol-miR-153a, pol-miR-26b and pol-miR-26a	Gu et al. (2014)
Oreochromis niloticus	miR-34c-5p, miR-153-5p, and miR-749	miR-1306-5p, miR-132b, and miR-18c	Xiao et al. (2014)
Takifugu rubripes	miR-145-5p, miR-202-5p, miR143-3p, miR-145b-5p, miR-100- 5p, miR-22a-3p, miR-125b-5p, miR-223-3p, miR-451-5p, miR199-5p	fru-miR-202-5p, fru-miR-24-3p, fru-miR-145b-5p, fru-miR-2478-3p and fru-miR-2898-3p	Wongwarangkana et al. (2015)
Danio rerio	miR-21, miR-92a, miR-10c, miR- 202-5p, let-7a, miR-27c-3p, miR- 25, miR-126a-3p, miR-26a, miR- 145	dre-let-7a, dre-miR-125a, dre-miR-132-3p, dre-miR-150, dre-miR-212, dre-miR-735, dre-miR-2187-5p, dre-miR-2189	Vaz et al. (2015)
Oreochromis niloticus	miR-727, miR-129, and miR-29	miR-132, miR-212, miR-33a, and miR-135b	Tao et al. (2016)
Oryzias melastigma	miR-21, miR-143-3p, miR-181, miR-100-5p, miR-26c, miR-181a- 5p, miR-143, let-7b, miR-10b-5p, miR-100-5p		Lai et al. (2016)
Oplegnathus punctatus	miR-92 family, let-7 family, opu-miR-25-3p, opu-miR-133a-3p, opu-miR-200a and opu-miR-429a	opu-miR-21, opu-miR-100-5p, opu-miR-10 and opu-miR-202-5p	Du et al. (2018)
Acipenser schrenckii	miR-203b-3p, miR-301a-5p, miR-146b-5p and miR-2779	miR-9b-5p, novel-28, miR-30d, miR-27e, let-7a-5p, miR-200b and miR-16-5p	Zhang et al. (2018)
Oryzias latipes	miR-143, mir-202-5p, miR-21, let-7a-5p, miR-26, miR-146a-5p, miR-30a-5p, miR-26, miR-181a- 5p, miR-22	miR-202-5p, pc-5p-214-47,91	Qiu et al. (2018)
Danio rerio	miR-22a-3p, miR-100-5p, miR- 20a-5p, let-7a, miR-26a-5p, miR- 92a-3p, miR-143, miR-9-5p, miR-99, miR-21, miR-22a-3p, let-7a, miR-100-5p, miR-143, miR-21, miR-202-5p, miR-99, miR-20a-5p, miR-26a- 5p, miR-181a-5p		Wong et al. (2018)
Oreochromis niloticus	miR-199a-3p, let-7c, miR-140-3p miR-100-5p, miR-146a-5p, let- 7a-5p, miR-143-3p, miR-21-5p,	miR-145–5p	Pinhal et al. (2018)
Cyprinus carpio	miR-101a, miR-199-5p	miR-143, miR-99, miR-101a, miR-100, miR-22a, miR-146a, miR-21, and miR-7a	Tao et al. (2018)
Danio rerio	miR-202-5p, miR-143, miR-22a- 3p, miR-92a-3p, miR-26a-5p, miR-181a-5p, miR-21, miR-30d, miR-27c-3p, miR-27b-3p		Zayed et al. (2019)
Trachinotus ovatus	dre-let-7c-5p, dre-miR727-5p, dre-miR-181a-5p, dre-miR-92a-3p, Novel miRNA 124, and Novel miRNA 190	dre-miR-7b, dre-miR-7a, dre-miR-143, dre-miR-101a, dre-miR-144-3p, dre-miR-202-5p, dremiR-153a-3p, and dre-miR-301a	He et al. (2019)
Acanthopagrus latus	miR-200, miR-29, miR-21, and miR-725	let-7, miR-10, miR-7, miR-9, and miR-202-3p	Li et al. (2020)
Salmo salar	miRs 92a-3p, 202-5p, 15c-5p, and 30d-5p	miR-15c-5p, miR-30d-5p, miR-93a-5p, and miR-730-5p	Bizuayehu and Babiak (2020)

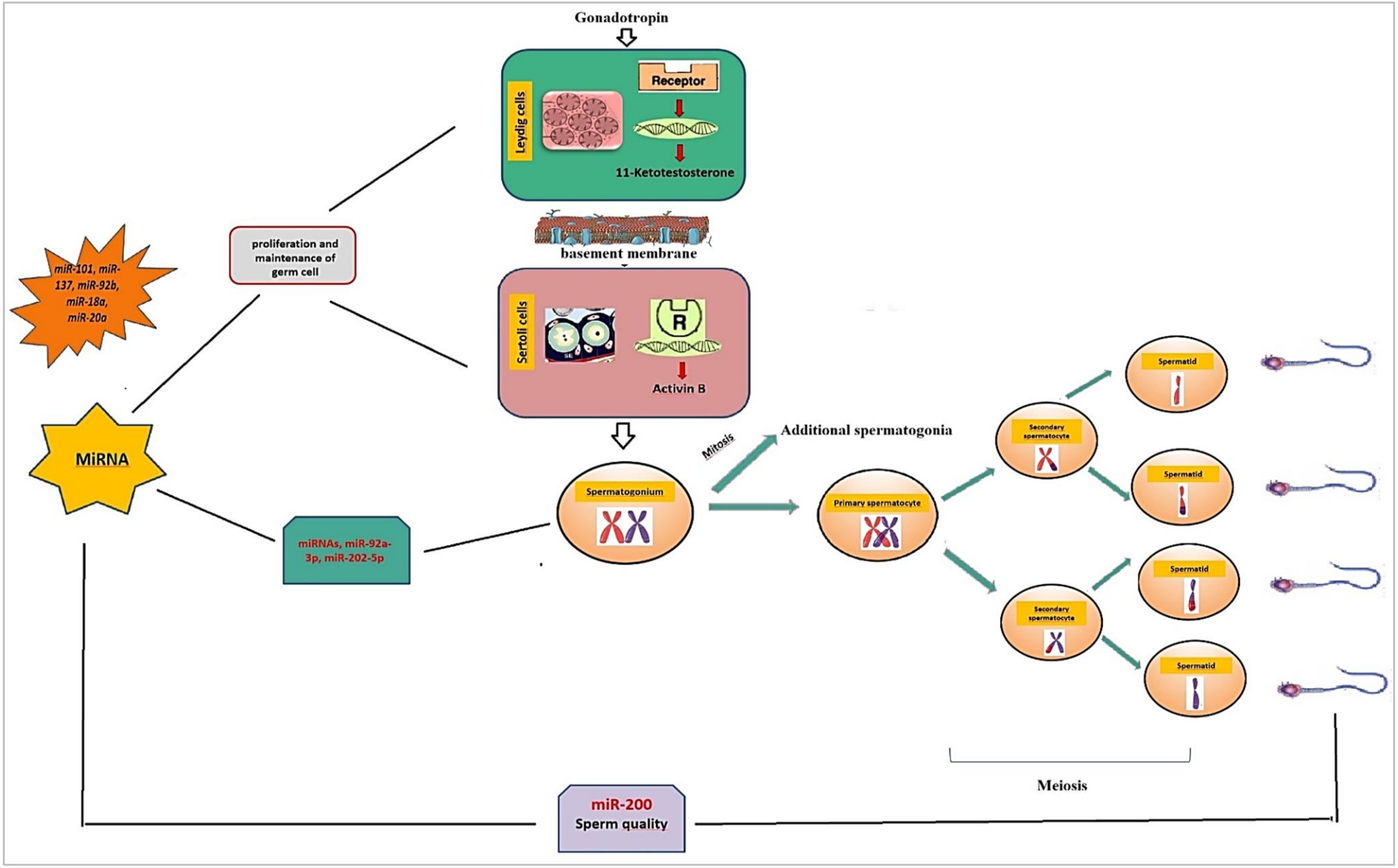

Figure 4. Schematic figure demonstrating the role of miRNA on fish spermatogenesis.

parameters directly related to the fertilization capacity of sperm can potentially be used for the evaluation of sperm quality. In research, sperm density, osmotic structure, pH of seminal fluid, chemical structure of sperm and seminal plasma, enzymatic activities, concentration of ATP (adenosine triphosphate), motility, morphological structure, fertilisation capacity, and other features are used to define the quality of sperm (Billard et al. 1995; Lansteiner et al. 1998; Fauvel et al. 1998; Geffen and Evans, 2000; Chowdhury and Joy, 2001).

Semen analysis has a great place in the assessment of male infertility. Molecular sperm parameters evaluation with miRNA could be used with traditional techniques (Figure 5). In this analysis, traditional semen parameters such as semen volume and pH, motility, morphology, and concentration of spermatozoa, are examined (Kutluyer Kocabaş, 2022). It has been revealed that miR-210-3p may play a role in sperm cell apoptosis by activating caspase-3 (Kaya, 2023). Glycolysis can provide energy for sperm cell motility via anaerobic respiration. MiRNAs are known to engage in the glycolytic process by regulating target genes. Reduced expression of let-7b-5p has been demonstrated to reduce glycolysis in asthenozoospermia through inhibiting AURKB (Wang et al., 2020). On the other hand, debates continue about the adequacy of these analyses in evaluating male infertility (Lewis, 2007), and studies

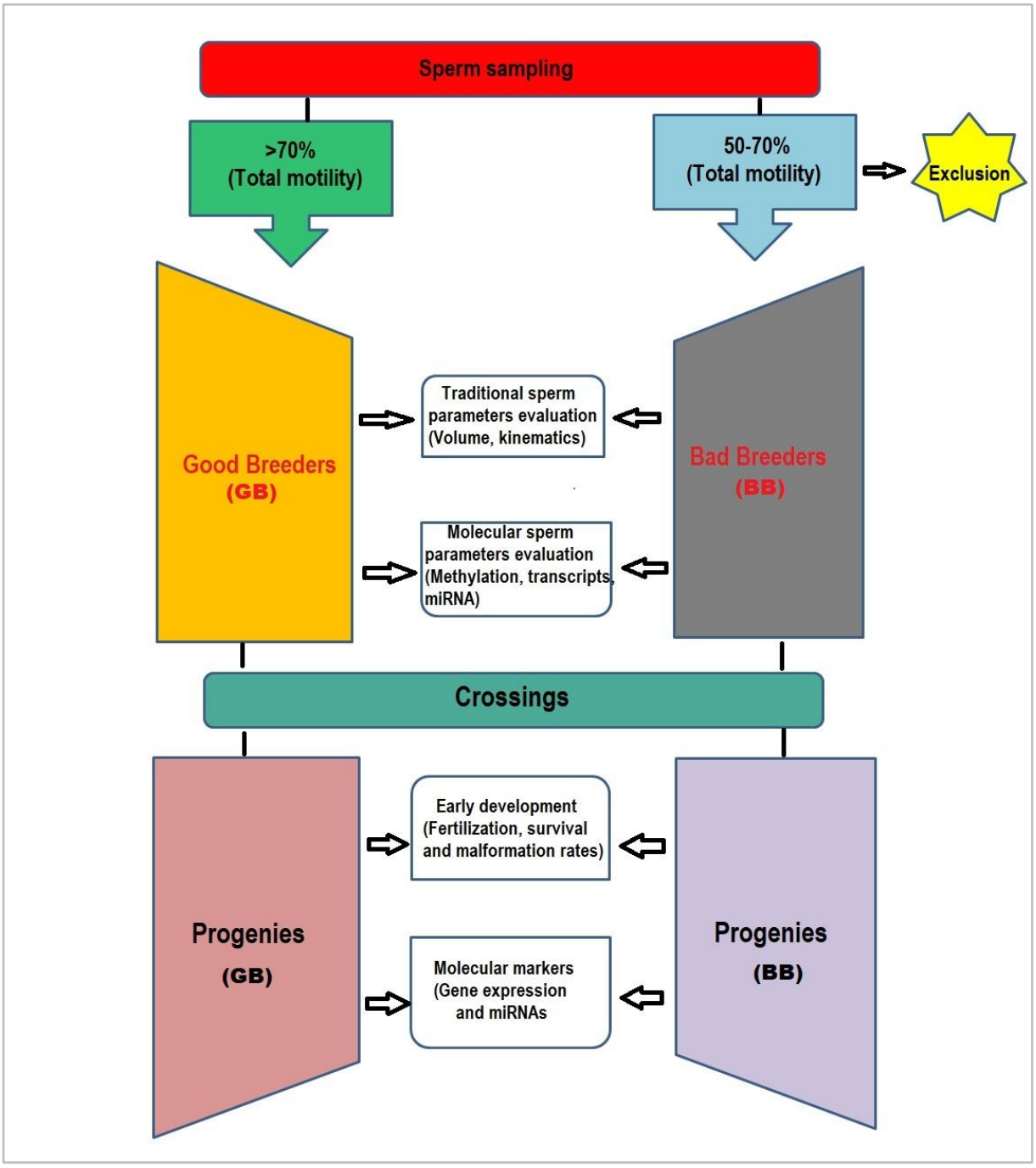

Figure 5. Schematic representation of sperm parameters evaluation methods adapted from Riesco et al. (2019)

are carried out to develop new fertility markers (Lalancette et al., 2009). Comparatively investigated spermatozoal RNAs between fertile and infertile individuals are among the recommended biomarkers (Jodar et al., 2012; Malcher et al., 2013; Garrido et al., 2013). Thus far, a few researches on miRNA have been performed in fish spermatozoa. miRNA profiling and abundance in zebrafish (*Danio rerio*) sperm have been documented by Jia et al. (2015) and Riesco et al. (2019). Amos (2008) stated that sperm motility and flagellar stability may linked with *Tekt1* (a member of the tektin family) through involvement in sperm flagellum formation. Xu et al. (2015) and Tao et al. (2018) reported downregulation of sperm motility and flagellar assembly by a series of important genes (*Dnahs, Dnal1, Ifts,* and *Dnaaf1*) expression in sterile triploids [*Carassius auratus* red var. × (*Carassius auratus* × *Cyprinus carpio*)] compared to fertile diploids (*Carassius auratus* red var.). Tao et al. (2018) showed these differentially expressed genes' impairment in gamete formation of triploid lines. Xiong et al. (2018) stated that sperm motility is regulated and improved in Zebrafish with an miR-200 Cluster on Chromosome 23. *wt1a*, *amh,* and *srd5a2b* are sperm motility-related genes and direct targets of miR-200s on chr23. In addition, they reported that the motility traits of sperm were reduced with ectopic expression of miR-200a, miR-200b, and miR-429a. Bizuayehu and Babiak (2020) have expressed miRNAs, miR-92a-3p and miR-202-5p in somatic supporting cells and spermatogonia in the immature testis of *Salmo salar*.

Conclusion and Perspectives

In aquaculture, reproductive success depends on high-quality gametes. As the key regulators, miRNAs affect gene expression, physiological processes, and fertilization success. In particular, repression and activation of maturation are linked with miRNA by suppressing mRNAs/pathways. Traditional techniques are insufficient to evaluate sperm quality parameters and male fertility potential. miRNAs regulate sperm quality and can be potential markers and provide some developments in alternative applications of miRNAs. The miRNA biogenesis pathway seems to be a potential target for understanding the mechanisms of different physiological events and sperm quality at the molecular level and alternative applications for enhancing global aquaculture production.

Ethical Statement

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Author Contribution

FKK and MK devised, structured, and wrote the manuscript. All the authors reviewed and corrected the manuscript.

Conflict of Interest

The authors declared no conflict of interest in this work. The author is responsible for the content and the writing of the paper.

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