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Role of MicroRNAs in Mammalian Reproduction

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ABSTRACT

MicroRNAs (miRNAs) are small, endogenous, non-coding RNA molecules that are around 19-25 nucleotides long. Most miRNAs are produced via transcription of DNA sequences into primary, precursor, and then mature miRNAs. These miRNAs molecules formed base-pair to mRNAs to post-transcriptionally drive gene expression. There are now over 2000 miRNAs annotated in the mammalian genome, which are thought to affect one-third of the genes, as each miRNA can regulate hundreds of target genes. miRNAs are involved in morphogenesis, tissue maintenance, cell development, differentiation, apoptosis, and metabolism etc. The aim of this review is to summarize the current knowledge on the role of miRNAs in various stages of mammalian reproductive biology and their involvement in reproductive disorders. We have also summarized the recent advances about miRNA and provide an updated overview of the literature, including the most recent and relevant studies.

Introduction

MicroRNAs (miRNAs) are small non-coding RNA molecules that play an important role in regulating gene expression. In recent years, the role of miRNAs in mammalian reproduction has gained much attention as they have proved to be important regulators of numerous aspects of reproductive biology, including gametogenesis, fertilization, implantation, and pregnancy (Hu et al., 2008; Ioannidis and Donadeu, 2016; Reza et al., 2019). They have also been implicated in the development of various reproductive disorders, such as infertility, pregnancy loss, and preterm birth. The discovery of miRNAs has led to a better understanding of the molecular mechanisms underlying reproductive biology and has opened new opportunities for the development of therapeutic interventions for reproductive disorders (Barranco et al., 2022). The ability of miRNAs to regulate multiple targets and pathways makes them attractive molecules for the development of novel therapeutic strategies.

MiRNAs, nuclear-derived non-coding biomolecules are becoming increasingly significant in modern molecular research. Mostly, miRNAs bind with the 3′ untranslated region (3′ UTR) of target mRNAs to trigger mRNA through degradation and translational suppression (Fatima et al., 2013). There are several numbers of mature miRNA reported in the farm animal species such as cattle (1025), sheep (153), goat (436), pig (457), chicken (1232) (Data source: MiRBase release 22 (http://www.mirbase.org/); PubMed databases). There are two types of pathways for the biological synthesis of miRNA- Canonical and Non-

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canonical pathways (Thorne et al., 2018). One is canonical pathway in which DiGeorge Syndrome Critical Region 8 (DGCR8) identifies the N6-methyladenylated GGAC group on pre-miRNA and hairpin structure of pre-miRNA cleaved by Drosha, a class-2 RNase III enzyme that converts the pre-miRNA into a precursor miRNA (pre-miRNA) inside the nucleus which in turn forms a 2 nucleotide 3' overhang of pre-miRNA. And then, exportin-5/Ran GTP (XPO5/Ran GTP) transport of pre-miRNAs to the cytoplasm (Vasiljevic et al., 2022). In cytoplasm RNase III endonuclease Dicer process, the removal of the terminal group then both strands of miRNA are loaded into Argonaut (AGO) protein group by the use of ATP. Preferably the strand having lower 5' stability is loaded to the AGO protein family (O'Brien et al., 2018). The unloaded strands are generally called the passenger strands having no mismatches, these passenger strands unwind from the guide strand and are cleaved by AGO2 and precede for biological degradation which results in a strong strand production. Second is Non-canonical pathway which can be Drosha/DCGR8 independent and Dicer-independent. In Drosha/DCGR8 independent pathway pre-miRNA (mirtons, 7-methylguanosine (m7G) -capped) resembles to Dicer substrate transported to the cytoplasm via XPO-1 without the Drosha cleavage. Strong 3' strand due to m7G cap prevents 5' strand loading into the protein AGO. In this pathway, short hairpin RNAs (shRNA) are processed into pre-miRNAs by Drosha where these pre-miRNAs need AGO2 for the maturation in the cytoplasm that enhances the loading of entire pre-miRNA into AGO2 protein slicing of 3' strand. Cutting of 3'-5' of the 5' strand completes the synthesis of mi-RNA (Ha et al., 2014).

Recently, the number of studies investigating the role of miRNAs in reproductive biology has increased significantly. The identification of miRNAs and their targets has provided a new perspective into the molecular mechanism that is behind the reproductive processes and has led to the identification of potential therapeutic targets and diagnostic biomarkers for the treatment and prediction of reproductive disorders. miRNAs have been shown to regulate the expression of genes involved in oocyte maturation and fertilization, as well as the blastocyst development and implantation (Kropp et al., 2015). However, there are still many challenges to be addressed before miRNA-based therapies can be successfully implemented in the clinic.

miRNA in reproduction

MicroRNAs play an important role in the development and operation of numerous reproductive processes, such as ovarian follicle formation, ovulation, spermatogenesis, pregnancy etc. Both male and female become infertile when one or both of the components of the miRNA-processing machinery (Dicer and Drosha) are lost; this is because there are significant flaws during gametogenesis. miRNAs are currently being studied extensively in bovine and other livestock, including testicular and ovarian tissues, embryonic and adipose tissues and the mammary gland (Fatima et al., 2013). Dicer and Drosha deletion in male primordial germ cells (PGCs) limits the growth, differentiation, and maturation of male PGCs, resulting in defective spermatozoa and sterility. In the testes, miRNAs have been shown to be involved in the regulation of spermatogenesis. Dicer1 was specifically deleted from Sertoli cells, which resulted in apoptosis of the Sertoli cells after birth and degenerating the testes, impairing pre-pubertal spermatogenesis, and causing infertility (Papaioannou et al., 2009).

One of the key functions of miRNAs in the female reproductive organs involves in the regulation of folliculogenesis, the process by which ovarian follicles develop and mature. miR-221/222 has been found to regulate the proliferation and differentiation of granulosa cells in the ovary (Gao et al., 2018), while miR-31 has been found to regulate the development of corpus luteum, a hormone-secreting structure that forms in the ovary after ovulation (Baddela et al., 2017). Dicer1 loss results in abnormal spindle formation and chromosomal alignment failures in meiotic maturation and polar body formation in females. It also leads to functional defects, such as impaired steroidogenesis and granulosa cell (GC) proliferation, recruitment of immature follicles, follicular atresia, a smaller pool size of pre-ovulatory follicles, and ovulatory dysfunction leading to premature ovulation (Reza et al., 2019). Some of the miRNA involved in male and female reproductive processes are described in Table 1.

miRNA in spermatogenesis

Spermatogenesis is a complex differentiation program that begins after birth when spermatogonial stem cells (SSCs) enter the differentiation pathway and subsequently undergo mitotic divisions to expand the colony of differentiating germ cells. miR-302 helps in the proliferation and differentiation of spermatogonial stem cells. miR-14 and miR-34 are important for meiosis and the development of the sperm tail, which is important for sperm motility (Kamkina et al., 2018). miRNAs help in spermatogenesis by regulating gene expression and ensuring the proper development of sperm. miRNA-375 has also been shown to play a role in spermatogenesis by regulating the expression of a protein called PRM2 (Protamine 2), which is involved in the formation of the acrosome, a structure on

Table 1: miRNAs involved in various reproductive processes

S. No.	Process	miRNAs involved	Reference
1.	Angiogenesis	miR-17-92 family; miR-17, -18a, -19a, -19b, -20a and -92a, miR-145, and miR-92a	Anand and Cheresh, 2011
2.	Gonadal development	miR-101, miR-202	Torley et al., 2011
3.	Spermatogenesis	miR-221, -203 and -34b-5p, miR-34b/c and miR-449	Wu et al., 2014
4.	Granulosa cell functioning	miR21, miR23a, miR145, miR503, miR224, miR383, miR378.	Donadeu et al., 2012
5.	Luteinization	miR-9 & let7b	Donadeu et al., 2012
6.	Luteal cell regression/ apoptosis	miR378	Ma et al., 2011
7.	Ovulation	miR-21, miR-23b, miR-378, miR-202 miR-145	Donadeu and Schauer, 2013
8.	Implantation	miR-143, miR-21, miR-20a, miR-26a, let-7a, let-7b, let-7c, and let-7d	Hu et al., 2008
9.	Placental function	miR-126, miR-125b, miR-92b, miR-106a, miR-24, and miR-20	Yan et al., 2014
10.	Pregnancy	hsa-miR-526a, hsa-miR-527 and hsa-miR-520d-5p, miR-26a	Ioannidis and Donadeu, 2016

the surface of the sperm that is necessary for fertilization (Reza et al., 2019). miRNA-29a help in the expression of a protein called HSP90AA1, which is involved in the maturation and storage of sperm (Richard et al., 2020). miRNA-200c has been found to regulate the sperm maturation by influencing the expression of a protein called SPAG9, which is involved in the organization of the cytoskeleton in sperm.

According to transcriptome data, miRNAs are significantly expressed during spermatogenesis. While a few miRNAs are exclusively expressed in certain types of male germ cells, others are frequently expressed in all types of testicular cells. Spermatogenesis, which in mammals has three stages, mitotic proliferation of spermatogonia, meiosis of spermatocytes, and haploid differentiation of spermatids, generates male gametes inside the testis. These procedures call for precisely controlled gene expression patterns that are regulated by post-transcriptional, transcriptional, and epigenetic processes (Papaioannou et al. 2010). While the other miRNAs are generically expressed across all types of testicular cells, few miRNAs are selectively expressed in specific types of male germ cells (Gao et al., 2019). Dicer1 elimination has been observed to alter meiotic progression, pachytene spermatocyte apoptosis, the number of round spermatids, and structural defects in spermatozoa in pro-spermatogonia prior to birth when Ddx4 promoter-driven Cre expression is used. SSC-enriched population preferentially expressed miR-20 along with miR-21, 34c, 135a, 146a, 182, 183, 204, 465a-3p, 465b-3p, 465c-5p, and 544 (He et al., 2013). When meiosis begins throughout the formation of the testis and in adult testes, the expression of the miR-449 cluster is numerous and elevated (Yuan et al., 2015). The testicular Sertoli cells, peritubular myoid (PTM) cells, and leydig cells all contribute to spermatogenesis (Zhou et al., 2019). The expression of Cyclin B1 and Cyclin D1 is regulated by miR-133b, which targets GLI3 and encourages the proliferation of human Sertoli cells (Yao et al., 2016). Many of the miRNAs functions and underlying processes in spermatogenesis are still substantially unclear. There is increasing evidence that certain miRNAs are expressed more frequently in testicular somatic cells. However, it is unclear whether these miRNAs function in the SSC niche as paracrine factors that are secreted or whether they instead indirectly regulate the production of growth factors like GDNF, that have an impact on germ cells.

miRNA in oogenesis

In oogenesis, miRNAs are known to play a critical role in various stages of folliculogenesis, including the regulation of follicle growth, meiotic maturation, and oocyte quality. miR-17 has been shown to play a role in follicle maturation by regulating the proliferation and differentiation of granulosa cells, which surround the developing oocyte (Inoue et al., 2020). In addition, miR-375 has been found to be important for oocyte maturation in mammals (Tesfaye et al., 2009). Folliculogenesis is the process of oocyte maturation, which is a critical process in female reproductive biology. The regulation of folliculogenesis is highly complex and involves the coordination of multiple molecular pathways. miRNAs are known to play a key role in the

regulation of several key pathways, including the PI3K/AKT and BMP (Bone Morphogenetic Protein) signaling pathways. miR-125b has been shown to regulate granulosa cell proliferation and differentiation by targeting the PI3K/AKT signaling pathway (Andrei et al., 2019). Additionally, miR-143, miR-145, and miR-503 have been shown to regulate ovarian follicle growth and oocyte maturation through the modulation of BMP (Bone Morphogenetic Proteins) signaling (Reza et al., 2019). Meiotic maturation is a complex process that involves the regulation of numerous molecular pathways. Several miRNAs that are differentially expressed during meiotic maturation, including miR-34c, miR-212, miR-222, and miR-223 and play a critical role in the regulation of meiotic maturation by targeting the cyclin B1 gene also involved in the regulation of meiotic maturation, although their specific targets are not yet well understood (Wanet et al., 2012). miR-21 protects oocytes from oxidative stress by targeting the PTEN (phosphatase and tensin homolog deleted on chromosome 10) gene. Additionally, miR-34a regulates oocyte apoptosis by targeting the Bcl-2 gene. miRNAs have also been implicated in the regulation of oocyte aging by targeting the SIRT1 gene which is involved in the regulation of aging and age-related diseases (Yamakuchi et al., 2008).

miRNA in fertilization

The process of fertilization is complex, which includes sperm-egg recognition, binding, and fusion. miR-34c has been shown to be present in the female reproductive tract and to be involved in regulating sperm-oocyte binding by targeting the integrin β1 gene in the oocyte (Bridi et al., 2020). Similarly, miR-122 is present in the sperm and plays a role in sperm-oocyte binding by regulating the expression of the zona pellucida glycoprotein 3 (ZP3) genes in the oocyte (Yang et al., 2013). miRNAs are also involved in regulating the sperm-oocyte binding like miR-34c affect the expression of the ion channel protein CatSper, which is required for sperm-oocyte fusion (Kathirvel et al., 2013). Similarly, miR-199a-5p has been shown to regulate the expression of the membrane protein CD9, which is also required for sperm-egg fusion (Chen et al., 2023). After fertilization, miRNAs are also involved in a series of cell divisions to form a blastocyst, which will eventually implant in the uterus. miR-34a is involved in regulating the cell cycle in the zygote by targeting the cyclin-dependent kinase 6 (CDK6) gene (Reza et al., 2019). Similarly, miR-21 helps in the proliferation and differentiation of embryonic stem cells (Ren et al., 2009). In addition to their roles in fertilization and early embryonic development, miRNAs have also been implicated in various reproductive disorders, including infertility, endometriosis, and polycystic ovary syndrome (PCOS) (Abdalla et al., 2020). miR-122 has been shown to be down-regulated in the sperm of infertile men, and miR-21 has been shown to be up-regulated in the endometrium with endometriosis (Bahmyari et al., 2021). Understanding the role of miRNAs in fertilization may provide new insights into the causes of infertility and other reproductive disorders.

miRNA in embryonic development

In IVF-produced embryos, higher levels of miR-130b expression were found at the morula and blastocyst stages, but suppression of this miRNA drastically decreased morula and blastocyst formation (Sinha et al., 2017) The role of miR-297, miR-96, miR-21, miR-29c, let-7, miR-214, miR-125a, miR-424, and miR-376a in trophectoderm specification has been speculated (Viswanathan et al., 2009). Human trophoblast cells' ability to migrate and invade has been shown to be regulated by miR-519d, miR-378a-5p, miR-376, and miR-155 (Xie et al., 2014). miRNAs are also thought to play a role in controlling embryo elongation. When compared to in vitro fertilized (IVF) embryos, in vivo fertilized embryos exhibit reduced blastocyst stage expression of miR-21 (Stowe et al., 2012). However increased miR-24 expression restricted embryos from growing to the blastocyst stage (Kropp et al., 2015). Because that miR-24 is extremely conserved among mammalian species, it might be used as a biomarker for the quality of the embryo. miR-199-5P is consistently down-regulated in embryos fertilized in vitro as compared to those fertilized in vivo during both the blastocyst and epiblast phases of embryo development. It has shown that the dysregulated miR-199-5P produced high glycolytic rates in blastocysts. These changes explained the difference between the development of in-vitro and in-vivo fertilized embryos by causing genetic imbalance and greater fetal losses (Tan et al., 2016). miR-29b enhances embryonic quality, lowers DNA methylation and apoptosis, and modifies gene expression in SCNT blastocysts without influencing blastocyst production. Treatment with miR-29b also enhanced the expression of pluripotency, development- and methylation-related genes in blastocysts (Singh et al., 2019). While miR-21 up-regulates the expression of the developmental and pluripotency-related genes (Rashmi et al., 2019).

Early gastrulation-stage embryos are regulated by miR-124a, miR-200a/b/c, miR-141, and miR-429 (Berardi et al., 2012). miR-191 and miR-16-1, which are numerous in embryonic stem cells (ESCs), inhibit the differentiation of ESCs towards the endoderm and ectoderm lineages by down-regulating Smad2, a key element of activin-nodal

signaling (Hadjimichael et al., 2016). In contrast, overexpression of miR-21 promotes the commitment of mesodermal tissues such as adipose and bone, while expression of miR-9, miR-124a, miR-155, and miR-708 stimulates the differentiation of ESCs (Reza et al., 2019). Similarly, by influencing Nodal signaling, the miR-290-295 and miR-303-367 clusters may be crucial for the patterning and determination of embryonic germ layers (Vidigal and Ventura, 2012).

Embryo implantation and development is a critical process for a successful pregnancy establishment. Several factors are involved in this process including miRNA, which play a vital role in the embryo implantation process. miRNAs are dynamically expressed during embryonic development, and their dysregulation can lead to developmental abnormalities (Xu et al., 2016). miR-430 has been shown to be essential for proper germ layer formation and embryonic development (Giraldez et al., 2006). In mammals, the miR-17-92 cluster expresses itself at a high level throughout the early stages of lung development but decreases as the process continues (Bonauer et al., 2009). Loss of miR-302/367 cluster, which is highly expressed in embryonic stem cells, leads to defects in embryonic development and differentiation (Sinkkonen et al., 2008). miRNAs also play an important role in neural development, cardiac development, limb development during embryogenesis. miR-124 is highly expressed in neuronal cells and has been shown to promote neuronal differentiation and suppress glial differentiation (Makeyev et al., 2007). Another miRNA, miR-9, has been shown to regulate neuronal migration and axon guidance during neural development (De Pietri Tonelli et al., 2008). Additionally, miR-17-92 cluster has been shown to be important for neural progenitor cell proliferation and differentiation (Krichevsky et al., 2006). The deletion of miR-1-2 leads to cardiac defects and embryonic lethality (Zhao et al., 2007). miR-499, which is highly expressed in the heart, has been shown to regulate cardiac differentiation and function (Wang et al., 2017). Additionally, miR-133 has been shown to regulate cardiac development and hypertrophy (Zhao et al., 2007). Additionally, miR-140 has been shown to regulate chondrogenesis during limb development (Tuddenham et al., 2006).

miRNA in pregnancy and placental development

miRNAs have also played important roles in pregnancy and placental development. The placenta is a vital organ that plays a crucial role in the exchange of nutrients, gases, and waste products between the mother and the developing fetus. Dysregulation of placental development has been associated with various pregnancy related complications like preterm birth. miR-210 was significantly up-regulated in the placenta and its knockdown resulted in reduced placental angiogenesis and impaired fetal growth (Hayder et al., 2022), also miR-210 found to be down-regulaed in case of preeclampsia (Frazier et al., 2020). Whereas miR-518c down-regulation leading to reduced placental angiogenesis and impaired fetal growth (Hemmatzadeh et al., 2020) and miR-29a has been shown to be down-regulated in the uterine lining with recurrent pregnancy loss and its restoration has been shown to improve uterine receptivity and pregnancy outcomes (Liu et al., 2021). Another important role of miR-21 has been found in the upregulation of uterine endometrium during the implantation phase of pregnancy and is thought to play a role in the regulation of uterine receptivity (Li et al., 2014).

Blastocyst apposition, adhesion to the uterine epithelium, and decidualization of the uterine stroma are all essential in early stages of pregnancy, miRNAs have been suggested as key molecules in the regulation of decidualization, among the several processes (Bidarimath et al., 2014). In early pregnancy, Drosha expression in a mouse uterus has spatiotemporal properties. At the time of implantation artificially induced decidua and decidual stromal cells contained a significant amount of Drosha. According to the stromal cell culture model, Drosha expression rapidly increased as decidualization proceeded (Zhang et al., 2016). Numerous miRNAs show patterns of spatial and temporal change in uterine endometrium, which may control different cellular events in the uterus. Placenta-specific miRNAs, such as miR-141 and miR-519d-3p (a member of the C19MC family), were differentially expressed in various developmental stages to meet the various regulatory demands of pregnancy because they regulate trophoblast cell proliferation, invasion, migration, and interaction between cells (Ospina-Prieto et al., 2016). Seven members of the C19MC cluster, miR-518b, miR-1323, miR-516b, miR-515-5p, miR-520h, miR-519d, and miR-526b, were significantly repressed in the placenta of people with fetal growth restriction (FGR). Four of these members, miR-518b, miR-1323, miR-520h, and miR-519d, were confirmed as FGR-associated placenta-specific miRNA (Higashijima et al., 2013). It has been discovered that placental insufficiency results in the suppression of miR-514. It was also demonstrated that the pleiomorphic adenoma gene 1 (PLAG1), a significant regulator of insulin-like growth factor 1 (IGF-1), which contributes to FGR, is a target of the placenta-specific miR-141 (Tang et al., 2013).

The regulatory requirement of the physiological change, such as inflammation and hypoxia, determines the

differential expression of miRNAs in the placenta throughout gestation (Challis et al., 2009). Let-7 controls the expression of IL-6 negatively and works downstream of the NF-B signalling pathway. Let-7 was shown to be strongly expressed in the placenta and amnion, indicating that placental inflammation may be regulated (Chan et al., 2013). Similar to this, miR-181a inhibits the TGF- signalling pathway's activation and boosts the synthesis of IL-6; as a result, higher placental miR-181a expression reduces the immunosuppressive effects of mesenchymal stem cells and leads to dysfunctional pregnancy (Liu et al., 2012). The miR-148/152 family, on the other hand, mediates immunological tolerance to promote a healthy pregnancy by adversely regulating innate immune responses (Liu et al., 2010). Numerous placental miRNAs have been found to control trophoblast and endothelial cell activity during pregnancy. To promote syncytiotrophoblast development, the miR-17-92 cluster's paralogs miR-106a363 and miR-106b25 were significantly down-regulated (Kumar et al., 2013)

In primary trophoblasts exposed to hypoxia, a group of seven placenta-associated miRNAs, including miR-93, miR-205, miR-224, miR-335, miR-424, miR-451, and miR-491, were differentially expressed, and miR-205 was verified to target an essential placental development factor called MED1 (Mouillet et al., 2010). Further, the regulation of mitochondrial electron transport and the rennin-angiotensin system's is responsive to hypoxia by placenta-associated miRNAs. Hypoxic placenta had higher levels of HIF-responsive miR-210, which negatively impacted the efficiency of the mitochondrial electron transport chain and energy metabolism (Colleoni et al., 2013).

Dysregulation of miRNA expression has been found to be associated with these disorders, and the identification of miRNAs as diagnostic biomarkers and potential therapeutic targets is an active area of research such as miR-21 has been found to be up-regulated in the sperm with idiopathic infertility and its inhibition has been shown to improve sperm motility and fertilization rates (Liu et al., 2012).

Therapeutic importance of miRNA in reproduction

The potential therapeutic use of miRNAs in reproductive medicine is an area of active research and development, with several studies demonstrating the ability of miRNAs to regulate various aspects of reproductive biology. One approach currently being explored is the delivery of miRNAs to specific tissues or cells using nanoparticles or other delivery vehicles. For example, delivery of miR-34a to the testes of infertile mammals has been shown to promote spermatogenesis (Heydari et al., 2021), while the delivery of miR-221/222 to the ovary may stimulate folliculogenesis and improve fertility (Jain et al., 2022). Apart from this in the prostate gland, miRNAs have been shown to be involved in the regulation of prostate development and the progression of prostate cancer. miR-141 regulates the expression of a protein called PTEN, which is a tumor suppressor that is frequently lost in prostate cancer (Sharma et al., 2019). miR-205 plays a role in the progression of prostate cancer by regulating the expression of a protein called E2F3, which is involved in cell proliferation (Chauhan et al., 2020). In addition, the use of miRNAs as biomarkers for the diagnosis and prognosis of reproductive disorders has also been explored, with some promising results. Measurement of miR-514 in maternal circulation has been proposed as a potential marker for placental insufficiency (Wang et al., 2018).

Other potential therapeutic applications of miRNAs in reproductive medicine include regulation of pregnancy and placental development. Overexpression of miR-199a-3p in mouse placenta has been shown to improve placental development and fetal growth (Timofeeva et al., 2021), while the knockdown of miR-29a in the mouse uterus improves uterine receptivity and implantation rates (Liu et al., 2017). In addition, miRNAs were also found to play a role in the regulating of fetal growth and development as miR-27a has been found to be upregulated in the fetal liver in response to nutrient deprivation, suggesting a role in the regulation of fetal growth (Gu et al., 2013). Overall, the therapeutic potential of miRNAs in reproductive medicine is still at an early stage of development, but their ability to regulate gene expression at the post-transcriptional level makes them a promising target for the development of novel therapies for reproductive disorders.

Conclusion

Expression of miRNA in mammalian cells can regulate the expression of functional genes. Hence, it is an important non-coding RNA molecule that regulates mammalian growth and development. miRNAs play a critical role in oogenesis by regulating proliferation, development and apoptotic gene expression to ensure the proper development of ova. Identification of specific miRNAs and their target genes involved in embryonic development may provide new insights into the underlying molecular mechanisms and potential therapeutic targets for developmental disorders. However, since the exact mechanisms by which miRNAs regulate the process of spermatogenesis and oogenesis are not yet known and further research is needed to investigate their role.

Conflict of interest

None of the authors have any conflict of interest to declare.

References

Abdalla M, Deshmukh H, Atkin SL, Sathyapalan T. miRNAs as a novel clinical biomarker and therapeutic targets in polycystic ovary syndrome (PCOS): A review. Life Sci. 2020;259:118174. doi: 10.1016/j.lfs.2020.118174.

Anand S, Cheresh DA. Emerging Role of Micro-RNAs in the Regulation of Angiogenesis. Genes Cancer. 2011;2(12):1134-8. doi: 10.1177/1947601911423032.

Andrei D, Nagy RA, van Montfoort A, Tietge U, Terpstra M, Kok K, van den Berg A, Hoek A, Kluiver J, Donker R. Differential miRNA Expression Profiles in Cumulus and Mural Granulosa Cells from Human Pre-ovulatory Follicles. Microrna. 2019;8(1):61-67. doi: 10.2174/2211536607666180912152618.

Baddela VS, Onteru SK, Singh D. A syntenic locus on buffalo chromosome 20: novel genomic hotspot for miRNAs involved in follicular-luteal transition. Funct Integr Genomics. 2017;17(2-3):321-334. doi: 10.1007/s10142-016-0535-7.

Bahmyari S, Jamali Z, Khatami SH, Vakili O, Roozitalab M, Savardashtaki A, Solati A, Mousavi P, Shabaninejad Z, Vakili S, Behrouj H, Ghasemi H, Movahedpour A. microRNAs in female infertility: An overview. Cell Biochem Funct. 2021;39(8):955-969. doi: 10.1002/cbf.3671.

Barranco I, Salas-Huetos A, Berlanga A, Spinaci M, Yeste M, Ribas-Maynou J. Involvement of extracellular vesicle-encapsulated miRNAs in human reproductive disorders: a systematic review. Reprod Fertil Dev. 2022;34(11):751-775. doi: 10.1071/RD21301.

Berardi E, Pues M, Thorrez L, Sampaolesi M. miRNAs in ESC differentiation. Am J Physiol Heart Circ Physiol. 2012;303(8):H931-9. doi: 10.1152/ajpheart.00338.2012.

Bidarimath M, Khalaj K, Wessels JM, Tayade C. MicroRNAs, immune cells and pregnancy. Cell Mol Immunol. 2014;11(6):538-47. doi: 10.1038/cmi.2014.45.

Bonauer A, Dimmeler S. The microRNA-17-92 cluster: still a miRacle? Cell Cycle. 2009;8(23):3866-73. doi: 10.4161/cc.8.23.9994.

Bridi A, Perecin F, Silveira JCD. Extracellular Vesicles Mediated Early Embryo-Maternal Interactions. Int J Mol Sci. 2020;21(3):1163. doi: 10.3390/ijms21031163.

Challis JR, Lockwood CJ, Myatt L, Norman JE, Strauss JF 3rd, Petraglia F. Inflammation and pregnancy. Reprod Sci. 2009;16(2):206-15. doi: 10.1177/1933719108329095.

Chan HW, Lappas M, Yee SW, Vaswani K, Mitchell MD, Rice GE. The expression of the let-7 miRNAs and Lin28 signalling pathway in human term gestational tissues. Placenta. 2013;34(5):443-8. doi: 10.1016/j.placenta.2013.02.008.

Chauhan N, Dhasmana A, Jaggi M, Chauhan SC, Yallapu MM. miR-205: A Potential Biomedicine for Cancer Therapy. Cells. 2020;9(9):1957. doi: 10.3390/cells9091957.

Chen C, Zhang Z, Gu X, Sheng X, Xiao L, Wang X. Exosomes: New regulators of reproductive development. Mater Today Bio. 2023;19:100608. doi: 10.1016/j.mtbio.2023.100608.

Colleoni F, Padmanabhan N, Yung HW, Watson ED, Cetin I, Tissot van Patot MC, Burton GJ, Murray AJ. Suppression of mitochondrial electron transport chain function in the hypoxic human placenta: a role for miRNA-210 and protein synthesis inhibition. PLoS One. 2013;8(1):e55194. doi: 10.1371/journal.pone.0055194.

De Pietri Tonelli D, Pulvers JN, Haffner C, Murchison EP, Hannon GJ, Huttner WB. miRNAs are essential for survival and differentiation of newborn neurons but not for expansion of neural progenitors during early neurogenesis in the mouse embryonic neocortex. Development. 2008;135(23):3911-21. doi: 10.1242/dev.025080.

Donadeu FX, Schauer SN, Sontakke SD. Involvement of miRNAs in ovarian follicular and luteal development. J Endocrinol. 2012;215(3):323-34. doi: 10.1530/JOE-12-0252.

Donadeu FX, Schauer SN. Differential miRNA expression between equine ovulatory and anovulatory follicles. Domest Anim Endocrinol. 2013;45(3):122-5. doi: 10.1016/j.domaniend.2013.06.006.

Fatima A, Morris DG. MicroRNAs in domestic livestock. Physiol Genomics. 2013;45(16):685-96. doi: 10.1152/physiolgenomics.00009.2013.

Frazier S, McBride MW, Mulvana H, Graham D. From animal models to patients: the role of placental microRNAs, miR-210, miR-126, and miR-148a/152 in preeclampsia. Clin Sci (Lond). 2020;134(8):1001-1025. doi: 10.1042/CS20200023.

Gao H, Wen H, Cao C, Dong D, Yang C, Xie S, Zhang J, Huang X, Huang X, Yuan S, Dong W. Overexpression of MicroRNA-10a in Germ Cells Causes Male Infertility by Targeting Rad51 in Mouse and Human. Front Physiol. 2019;10:765. doi: 10.3389/fphys.2019.00765.

Gao K, Wang P, Peng J, Xue J, Chen K, Song Y, Wang J, Li G, An X, Cao B. Regulation and function of runt-related transcription factors (RUNX1 and RUNX2) in goat granulosa cells. J Steroid Biochem Mol Biol. 2018;181:98-108. doi: 10.1016/j.jsbmb.2018.04.002.

Giraldez AJ, Mishima Y, Rihel J, Grocock RJ, Van Dongen S, Inoue K, Enright AJ, Schier AF. Zebrafish MiR-430 promotes

deadenylation and clearance of maternal mRNAs. Science. 2006;312(5770):75-9. doi: 10.1126/science.1122689.

Gu Y, Sun J, Groome LJ, Wang Y. Differential miRNA expression profiles between the first and third trimester human placentas. Am J Physiol Endocrinol Metab. 2013;304(8):E836-43. doi: 10.1152/ajpendo.00660.2012.

Ha M, Kim VN. Regulation of microRNA biogenesis. Nat Rev Mol Cell Biol. 2014;15(8):509-24. doi: 10.1038/nrm3838.

Hadjimichael C, Nikolaou C, Papamatheakis J, Kretsovali A. MicroRNAs for Fine-Tuning of Mouse Embryonic Stem Cell Fate Decision through Regulation of TGF-β Signaling. Stem Cell Reports. 2016;6(3):292-301. doi: 10.1016/j.stemcr.2016.01.004.

Hayder HY. The role of miR-218-5p and miR-210-3p in human trophoblast function and placenta development., PhD Dissertation. York University, Toronto, Ontario. 2022.

He Z, Jiang J, Kokkinaki M, Tang L, Zeng W, Gallicano I, Dobrinski I, Dym M. MiRNA-20 and mirna-106a regulate spermatogonial stem cell renewal at the post-transcriptional level via targeting STAT3 and Ccnd1. Stem Cells. 2013;31(10):2205-17. doi: 10.1002/stem.1474.

Hemmatzadeh M, Shomali N, Yousefzadeh Y, Mohammadi H, Ghasemzadeh A, Yousefi M. MicroRNAs: Small molecules with a large impact on pre-eclampsia. J Cell Physiol. 2020;235(4):3235-3248. doi: 10.1002/jcp.29286.

Heydari H, Ghiasi R, Hamidian G, Ghaderpour S, Keyhanmanesh R. Voluntary exercise improves sperm parameters in high fat diet receiving rats through alteration in testicular oxidative stress, mir-34a/SIRT1/p53 and apoptosis. Horm Mol Biol Clin Investig. 2021;42(3):253-263. doi: 10.1515/hmbci-2020-0085.

Higashijima A, Miura K, Mishima H, Kinoshita A, Jo O, Abe S, Hasegawa Y, Miura S, Yamasaki K, Yoshida A, Yoshiura K, Masuzaki H. Characterization of placenta-specific microRNAs in fetal growth restriction pregnancy. Prenat Diagn. 2013;33(3):214-22. doi: 10.1002/pd.4045.

Hu SJ, Ren G, Liu JL, Zhao ZA, Yu YS, Su RW, Ma XH, Ni H, Lei W, Yang ZM. MicroRNA expression and regulation in mouse uterus during embryo implantation. J Biol Chem. 2008;283(34):23473-84. doi: 10.1074/jbc.M800406200.

Inoue Y, Munakata Y, Shinozawa A, Kawahara-Miki R, Shirasuna K, Iwata H. Prediction of major microRNAs in follicular fluid regulating porcine oocyte development. J Assist Reprod Genet. 2020;37(10):2569-2579. doi: 10.1007/s10815-020-01909-0.

Ioannidis J, Donadeu FX. Circulating miRNA signatures of early pregnancy in cattle. BMC Genomics. 2016;17:184. doi: 10.1186/s12864-016-2529-1.

Jain N, Gupta P, Sahoo S, Mallick B. Non-coding RNAs and their cross-talks impacting reproductive health of women. Wiley Interdiscip Rev RNA. 2022;13(3):e1695. doi: 10.1002/wrna.1695.

Kamkina P. Genetic and molecular characterization of new genes which influence germline apoptosis in C. elegans. Ph.D. Dissertation, University of Zurich. 2018.

Kathirvel P, Ramesh KG, Sankaranarayanan K. A computational prediction of conserved microRNA targets of ion channels in vertebrates. Current Bioinformatics. 2013;8(1):93-111.

Krichevsky AM, Sonntag KC, Isacson O, Kosik KS. Specific microRNAs modulate embryonic stem cell-derived neurogenesis. Stem Cells. 2006;24(4):857-64. doi: 10.1634/stemcells.2005-0441.

Kropp J, Khatib H. Characterization of microRNA in bovine in vitro culture media associated with embryo quality and development. J Dairy Sci. 2015;98(9):6552-63. doi: 10.3168/jds.2015-9510.

Kumar P, Luo Y, Tudela C, Alexander JM, Mendelson CR. The c-Myc-regulated microRNA-17~92 (miR-17~92) and miR-106a~363 clusters target hCYP19A1 and hGCM1 to inhibit human trophoblast differentiation. Mol Cell Biol. 2013;33(9):1782-96. doi: 10.1128/MCB.01228-12.

Li Y, Zhang J, Wei W, Wang Z, Prinz M, Hou Y. A strategy for co-analysis of microRNAs and DNA. Forensic Sci Int Genet. 2014;12:24-9. doi: 10.1016/j.fsigen.2014.04.011.

Liu A, Jin M, Xie L, Jing M, Zhou Y, Tang M, Lin T, Wang D. Loss of miR-29a impairs decidualization of endometrial stromal cells by TET3 mediated demethylation of Col1A1 promoter. iScience. 2021;24(9):103065. doi: 10.1016/j.isci.2021.103065.

Liu L, Wang Y, Fan H, Zhao X, Liu D, Hu Y, Kidd AR 3rd, Bao J, Hou Y. MicroRNA-181a regulates local immune balance by inhibiting proliferation and immunosuppressive properties of mesenchymal stem cells. Stem Cells. 2012;30(8):1756-70. doi: 10.1002/stem.1156.

Liu X, Zhan Z, Xu L, Ma F, Li D, Guo Z, Li N, Cao X. MicroRNA-148/152 impair innate response and antigen presentation of TLR-triggered dendritic cells by targeting CaMKIIα. J Immunol. 2010;185(12):7244-51. doi: 10.4049/jimmunol.1001573.

Liu Y, Ding Z. Obesity, a serious etiologic factor for male subfertility in modern society. Reproduction. 2017;154(4):R123-R131. doi: 10.1530/REP-17-0161.

Ma T, Jiang H, Gao Y, Zhao Y, Dai L, Xiong Q, Xu Y, Zhao Z, Zhang J. Microarray analysis of differentially expressed microRNAs in non-regressed and regressed bovine corpus luteum tissue; microRNA-378 may suppress luteal cell

apoptosis by targeting the interferon gamma receptor 1 gene. J Appl Genet. 2011;52(4):481-6. doi: 10.1007/s13353-011-0055-z.

Makeyev EV, Zhang J, Carrasco MA, Maniatis T. The MicroRNA miR-124 promotes neuronal differentiation by triggering brain-specific alternative pre-mRNA splicing. Mol Cell. 2007;27(3):435-48. doi: 10.1016/j.molcel.2007.07.015.

Mouillet JF, Chu T, Hubel CA, Nelson DM, Parks WT, Sadovsky Y. The levels of hypoxia-regulated microRNAs in plasma of pregnant women with fetal growth restriction. Placenta. 2010;31(9):781-4. doi: 10.1016/j.placenta.2010.07.001.

O'Brien J, Hayder H, Zayed Y, Peng C. Overview of MicroRNA Biogenesis, Mechanisms of Actions, and Circulation. Front Endocrinol (Lausanne). 2018;9:402. doi: 10.3389/fendo.2018.00402.

Ospina-Prieto S, Chaiwangyen W, Herrmann J, Groten T, Schleussner E, Markert UR, Morales-Prieto DM. MicroRNA-141 is upregulated in preeclamptic placentae and regulates trophoblast invasion and intercellular communication. Transl Res. 2016;172:61-72. doi: 10.1016/j.trsl.2016.02.012.

Papaioannou MD, Nef S. microRNAs in the testis: building up male fertility. J Androl. 2010;31(1):26-33. doi: 10.2164/jandrol.109.008128.

Papaioannou MD, Pitetti JL, Ro S, Park C, Aubry F, Schaad O, Vejnar CE, Kühne F, Descombes P, Zdobnov EM, McManus MT, Guillou F, Harfe BD, Yan W, Jégou B, Nef S. Sertoli cell Dicer is essential for spermatogenesis in mice. Dev Biol. 2009 326(1):250-9. doi: 10.1016/j.ydbio.2008.11.011.

Rashmi, Sah S, Shyam S, Singh MK, Palta P. Treatment of buffalo (Bubalus bubalis) SCNT embryos with microRNA-21 mimic improves their quality and alters gene expression but does not affect their developmental competence. Theriogenology. 2019 126:8-16. doi: 10.1016/j.theriogenology.2018.11.025.

Ren W, Qiang C, Gao L, Li SM, Zhang LM, Wang XL, Dong JW, Chen C, Liu CY, Zhi KQ. Circulating microRNA-21 (MIR-21) and phosphatase and tensin homolog (PTEN) are promising novel biomarkers for detection of oral squamous cell carcinoma. Biomarkers. 2014;19(7):590-6. doi: 10.3109/1354750X.2014.955059.

Reza AMMT, Choi YJ, Han SG, Song H, Park C, Hong K, Kim JH. Roles of microRNAs in mammalian reproduction: from the commitment of germ cells to peri-implantation embryos. Biol Rev Camb Philos Soc. 2019;94(2):415-438. doi: 10.1111/brv.12459.

Richard AJ, White U, Elks CM, Stephens JM et al., Adipose tissue: physiology to metabolic dysfunction. Endotext. 2020. PMID: 32255578.

Sharma N, Baruah MM. The microRNA signatures: aberrantly expressed miRNAs in prostate cancer. Clin Transl Oncol. 2019;21(2):126-144. doi: 10.1007/s12094-018-1910-8.

Singh S, Shyam S, Sah S, Singh MK, Palta P. Treatment of buffalo (Bubalus bubalis) somatic cell nuclear transfer embryos with microRNA-29b mimic improves their quality, reduces DNA methylation, and changes gene expression without affecting their developmental competence. Cell Reprogram. 2019;21(4):210-219. doi: 10.1089/cell.2019.0007.

Sinha PB, Tesfaye D, Rings F, Hossien M, Hoelker M, Held E, Neuhoff C, Tholen E, Schellander K, Salilew-Wondim D. MicroRNA-130b is involved in bovine granulosa and cumulus cells function, oocyte maturation and blastocyst formation. J Ovarian Res. 2017;10(1):37. doi: 10.1186/s13048-017-0336-1.

Sinkkonen L, Hugenschmidt T, Berninger P, Gaidatzis D, Mohn F, Artus-Revel CG, Zavolan M, Svoboda P, Filipowicz W. MicroRNAs control de novo DNA methylation through regulation of transcriptional repressors in mouse embryonic stem cells. Nat Struct Mol Biol. 2008;15(3):259-67. doi: 10.1038/nsmb.1391.

Stowe HM, Curry E, Calcatera SM, Krisher RL, Paczkowski M, Pratt SL. Cloning and expression of porcine Dicer and the impact of developmental stage and culture conditions on MicroRNA expression in porcine embryos. Gene. 2012;501(2):198-205. doi: 10.1016/j.gene.2012.03.058.

Tan K, Wang X, Zhang Z, Miao K, Yu Y, An L, Tian J. Downregulation of miR-199a-5p Disrupts the Developmental Potential of In Vitro-Fertilized Mouse Blastocysts. Biol Reprod. 2016;95(3):54. doi: 10.1095/biolreprod.116.141051.

Tang Q, Wu W, Xu X, Huang L, Gao Q, Chen H, Sun H, Xia Y, Sha J, Wang X, Chen D, Xu Q. miR-141 contributes to fetal growth restriction by regulating PLAG1 expression. PLoS One. 2013;8(3):e58737. doi: 10.1371/journal.pone.0058737.

Tesfaye D, Worku D, Rings F, Phatsara C, Tholen E, Schellander K, Hoelker M. Identification and expression profiling of microRNAs during bovine oocyte maturation using heterologous approach. Mol Reprod Dev. 2009;76(7):665-77. doi: 10.1002/mrd.21005.

Thorne JL, Battaglia S, Baxter DE, Hayes JL, Hutchinson SA, Jana S, Millican-Slater RA, Smith L, Teske MC, Wastall LM, Hughes TA. MiR-19b non-canonical binding is directed by HuR and confers chemosensitivity through regulation of P-glycoprotein in breast cancer. Biochim Biophys Acta Gene Regul Mech. 2018;1861(11):996-1006. doi: 10.1016/j.bbagrm.2018.08.005.

Timofeeva AV, Fedorov IS, Brzhozovskiy AG, Bugrova AE, Chagovets VV, Volochaeva MV, Starodubtseva NL,

Frankevich VE, Nikolaev EN, Shmakov RG, Sukhikh GT. miRNAs and Their Gene Targets-A Clue to Differentiate Pregnancies with Small for Gestational Age Newborns, Intrauterine Growth Restriction, and Preeclampsia. Diagnostics (Basel). 2021;11(4):729. doi: 10.3390/diagnostics11040729.

Torley KJ, da Silveira JC, Smith P, Anthony RV, Veeramachaneni DN, Winger QA, Bouma GJ. Expression of miRNAs in ovine fetal gonads: potential role in gonadal differentiation. Reprod Biol Endocrinol. 2011;9:2. doi: 10.1186/1477-7827-9-2.

Tuddenham L, Wheeler G, Ntounia-Fousara S, Waters J, Hajihosseini MK, Clark I, Dalmay T. The cartilage specific microRNA-140 targets histone deacetylase 4 in mouse cells. FEBS Lett. 2006;580(17):4214-7. doi: 10.1016/j.febslet.2006.06.080.

Vasiljevic A. Histopathology and molecular pathology of pediatric pineal parenchymal tumors. Childs Nerv Syst. 2022. doi: 10.1007/s00381-022-05637-x.

Vidigal JA, Ventura A. Embryonic stem cell miRNAs and their roles in development and disease. Semin Cancer Biol. 2012;22(5-6):428-36. doi: 10.1016/j.semcancer.2012.04.009.

Viswanathan SR, Mermel CH, Lu J, Lu CW, Golub TR, Daley GQ. microRNA expression during trophectoderm specification. PLoS One. 2009;4(7):e6143. doi: 10.1371/journal.pone.0006143.

Wanet A, Tacheny A, Arnould T, Renard P. miR-212/132 expression and functions: within and beyond the neuronal compartment. Nucleic Acids Res. 2012;40(11):4742-53. doi: 10.1093/nar/gks151.

Wang XY, Chen XL, Huang ZQ, Chen DW, Yu B, He J, Luo JQ, Luo YH, Chen H, Zheng P, Yu J. MicroRNA-499-5p regulates porcine myofiber specification by controlling Sox6 expression. Animal. 2017;11(12):2268-2274. doi: 10.1017/S1751731117001008.

Wang Y, Lumbers ER, Arthurs AL, Corbisier de Meaultsart C, Mathe A, Avery-Kiejda KA, Roberts CT, Pipkin FB, Marques FZ, Morris BJ, Pringle KG. Regulation of the human placental (pro)renin receptor-prorenin-angiotensin system by microRNAs. Mol Hum Reprod. 2018;24(9):453-464. doi: 10.1093/molehr/gay031.

Wu J, Bao J, Kim M, Yuan S, Tang C, Zheng H, Mastick GS, Xu C, Yan W. Two miRNA clusters, miR-34b/c and miR-449, are essential for normal brain development, motile ciliogenesis, and spermatogenesis. Proc Natl Acad Sci U S A. 2014 111(28):E2851-7. doi: 10.1073/pnas.1407777111.

Xie L, Mouillet JF, Chu T, Parks WT, Sadovsky E, Knöfler M, Sadovsky Y. C19MC microRNAs regulate the migration of human trophoblasts. Endocrinology. 2014;155(12):4975-85. doi: 10.1210/en.2014-1501.

Xu J, Feng L, Han Z, Li Y, Wu A, Shao T, Ding N, Li L, Deng W, Di X, Wang J, Zhang L, Li X, Zhang K, Cheng S. Extensive ceRNA-ceRNA interaction networks mediated by miRNAs regulate development in multiple rhesus tissues. Nucleic Acids Res. 2016;44(19):9438-9451. doi: 10.1093/nar/gkw587.

Yamakuchi M, Ferlito M, Lowenstein CJ. miR-34a repression of SIRT1 regulates apoptosis. Proc Natl Acad Sci U S A. 2008;105(36):13421-6. doi: 10.1073/pnas.0801613105.

Yan T, Cui K, Huang X, Ding S, Zheng Y, Luo Q, Liu X, Zou L. Assessment of therapeutic efficacy of miR-126 with contrast-enhanced ultrasound in preeclampsia rats. Placenta. 2014;35(1):23-9. doi: 10.1016/j.placenta.2013.10.017.

Yang S, Wang S, Luo A, Ding T, Lai Z, Shen W, Ma X, Cao C, Shi L, Jiang J, Rong F, Ma L, Tian Y, Du X, Lu Y, Li Y, Wang S. Expression patterns and regulatory functions of microRNAs during the initiation of primordial follicle development in the neonatal mouse ovary. Biol Reprod. 2013;89(5):126. doi: 10.1095/biolreprod.113.107730.

Yao C, Sun M, Yuan Q, Niu M, Chen Z, Hou J, Wang H, Wen L, Liu Y, Li Z, He Z. MiRNA-133b promotes the proliferation of human Sertoli cells through targeting GLI3. Oncotarget. 2016;7(3):2201-19. doi: 10.18632/oncotarget.6876.

Yuan S, Tang C, Zhang Y, Wu J, Bao J, Zheng H, Xu C, Yan W. mir-34b/c and mir-449a/b/c are required for spermatogenesis, but not for the first cleavage division in mice. Biol Open. 2015;4(2):212-23. doi: 10.1242/bio.201410959.

Zhang C, Long X, Ding Y, Chen X, He J, Liu S, Geng Y, Wang Y, Liu X. Expression of DROSHA in the Uterus of Mice in Early Pregnancy and Its Potential Significance During Embryo Implantation. Reprod Sci. 2016;23(2):154-62. doi: 10.1177/1933719115584444.

Zhao Y, Ransom JF, Li A, Vedantham V, von Drehle M, Muth AN, Tsuchihashi T, McManus MT, Schwartz RJ, Srivastava D. Dysregulation of cardiogenesis, cardiac conduction, and cell cycle in mice lacking miRNA-1-2. Cell. 2007 Apr 20;129(2):303-17. doi: 10.1016/j.cell.2007.03.030.

Zhou R, Wu J, Liu B, Jiang Y, Chen W, Li J, He Q, He Z. The roles and mechanisms of Leydig cells and myoid cells in regulating spermatogenesis. Cell Mol Life Sci. 2019;76(14):2681-2695. doi: 10.1007/s00018-019-03101-9.