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Opinions and Hypotheses

Is transcription in sperm stationary or dynamic?

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Abstract. Transcriptional activity is repressed due to the packaging of sperm chromatins during spermiogenesis. The detection of numerous transcripts in sperm, however, raises the question whether transcriptional events exist in sperm, which has been the central focus of the recent studies. To summarize the transcriptional activity during spermiogenesis and in sperm, we reviewed the documents on transcript differences during spermiogenesis, in sperm with differential motility, before and after capacitation and cryopreservation. This will lay a theoretical foundation for studying the mechanism(s) of gene expression in sperm, and would be invaluable in making better use of animal sires and developing reproductive control technologies.
Key words: Cryopreservation, Spermatozoa, Spermiogenesis, Transcriptional activity

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The development of haploid spermatids into mature spermatozoa requires a lengthy duration and entails a series of complex physiological changes. During spermiogenesis, the spermatid-specific H2B variants are specifically synthesized and expressed in round spermatids to replace the canonical histones. H2B variants have the capacity to open chromatin and form unstable nucleosomes, which facilitates histone acetylation; In the enlongated spermatids, the Brdt proteins combined with hyperacetylation histone mediate the histones removal, leading to the replacement of histones by transition proteins. Finally, the transition proteins are replaced by the smaller protamines [1–4]. In this way, the chromatin of sperm is condensed gradually and develops ultimately into a highly concentrated structure [5–13]. At the same time, the cytoplasm and ribosome of sperms slowly disappeared, accompanying the gradual differentiation of the other organelles, such as mitochondria and Golgi apparatus. As a result, spermatids become elongated cells and develop into tadpole-like cells with a head and tail. They enter the epididymis for maturation and eventually become sperms capable of movement with the potential for fertilization [6, 14]. This ordered maturation process is completed within a single sperm cell under conditions that exist in testis and epididymis, suggesting that gene transcription and translation play an important role in the regulation of this process. Since condensation of chromosomes occurs in spermatids, many scholars believe that transcription is terminated gradually with the compaction of chromosomal structure, presumably no transcription present in mature sperm [15–18]. For example, the transcription of few genes was detected in post meiosis phase in Drosophila [19], and the transcription was even undetected in late spermatids in mouse [20]. However, an increasing number of studies showed that sperm carry thousands of different types of RNA, including messenger RNAs (mRNA), microRNAs (miRNA), interference RNAs (IRNA), antisense RNAs, etc. [8, 11, 20–30]. In fact, more than 4,000 kinds of mRNAs were found in the studies of human sperm [11, 20, 31]. Due to the belief that gene transcription is silenced in sperm, the large quantity of RNA that nevertheless still remains is therefore hypothesized to exist as relics of spermatogenesis [11, 32]. Before terminating of nuclear transcription, the various mRNAs needed during the stages of spermiogenesis are transcribed in advance and retained for a long period of time; the mRNAs are then translated into proteins to ensure that all functions subsequent to nuclear transcription are normal and continuing [33–35]. Concerning that this hypothesis cannot explain the fact that a large number of rRNAs in the cytoplasm are removed or degraded, there are certainly different types of mRNAs left behind. In addition, studies also showed that the histones are not completely removed from nucleosomes in ejaculate spermatozoa, and they contributed to sperm chromatin approximately accounting for 1% in mouse [36], 15% in human [37], and 50% in some marsupial species [38]. As a result, some chromosomal regions of sperm manifest slacker conformations for the retained histones [17, 39], which may allow transcription factors to bind to specific gene sequences, providing transcriptional potential [35, 40–42]. In addition, a reverse transcriptase activity was observed in murine epididymal spermatozoa [43]. Here are the questions: are sperm RNAs the remnants from spermatogenesis before the end of nuclear

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transcription, or is there timely expression from sperm chromosomes; or do both occur? In order to reveal the nature of sperm maturation, improve the reproduction capability, and realize male contraception, the study on this topic is becoming more and more important.

However, the transcript number in sperm is relatively low [44], and all types of the germ line cells mix together. It is difficult to capture cell samples and obtain enough RNA samples for exploring the dynamics of sperm transcription. Currently, the combination of the technology of frozen sections and laser capture microdissection (LCM) overcomes the difficulty of sampling spermatids from different developmental stages during spermiogenesis [45, 46]. In addition, RNA amplification and RNA-Seq are now widely applied. The development of all these techniques is expecting to promote the discovery of the theme.

The transcripts in spermatids vary during post-meiotic

After meiosis, there are some morphological changes, including nuclear shaping and chromatin compaction as well as major cytoplasmic transformations in sperm [47, 48]. These post-meiotic events are considered to be driven by translation, as transcripts are considered originating from primary spermatocytes and stored in spermatids and translated during elongation. However, transcripts were detected in round spermatids [49] and elongated spermatids [50], and transcription of ram sperm chromatin was also examined by two electron microscopic techniques [51]. In 2006, Welch *et al.* analyzed the mRNA expression of glyceraldehyde-3-phosphate dehydrogenase gene (*Gapds*) in rat spermatogenic cells at different maturational time-periods using northern blot. They detected *Gapds* mRNA in round and condensing spermatids but not in primary spermatocytes [52], and demonstrated that the spermatogenetic cell-specific *Gapds* gene is inactivated in primary spermatocytes, whereas is expressed in the postmeiotic phase of spermatogenesis, and number of *Gapds* transcripts in condensing spermatids was significantly greater than in round spermatids. The detection of 24 comet and cup genes' transcripts during Drosophila spermatogenesis and spermiogenesis using *in situ* hybridization showed that the transcript number for hale-bopp (hale), schumacher-levy (schuy), davis-cup (d-cup), presidents-cup (p-cup), tetleys-cup (t-cup), flyer-cup (f-cup), sungrazer (sunz), and other genes in elongated spermatids was significantly higher than that of in round spermatids. In addition, the transcript number for these genes during the transformation of histones to protamine including complete replacement of histones by protamine shows a significant upward trend, which was proved by Q-RT-PCR [50]. According to post-meiotic transcription of these genes, authors drew a conclusion that transcription in Drosophila stops in late primary spermatocytes, then is reactivated by two pathways for a few loci just before histone-to-transition protein-to-protamine chromatin remodeling in spermiogenesis. Moreover, a surprisingly strong 5-bromouridine (BrU) signal was observed near spermatid nuclei in developing spermatid bundles during postmeiosis, and the BrU signal was reduced in the presence of actinomycin D, a general inhibitor of RNA synthesis [53]. They implied that the BrU signal in spermatids was dependent on RNA synthesis. Study showed that there are two categories of post-meiotic transcriptional regulation: methylation and trans-acting factors that bind to the TATA-box, the CRE-box, or other specific DNA sequence in the promoter region of nucleoproteins [49]. Since these genes are trancriptionally active only before the chromatin remodeling, how will the transriptional activity be after the histones are replaced by the protamines? It is still a highly debatable issue.

The transcripts in sperm vary with different sperm motilities

Motility is necessary for sperms to be able to penetrate cervical mucus, enter the fallopian tube, and eventually bind to the oocyte. Since the motility of sperms may vary among different animals and even among different sperms from the same sire, here we focus upon transcript variation among sperms with different motilities. The androgens/estrogens balance is essential for normal sexual development and reproduction in mammals. The P450 aromatase (P450arom) encoded by *cyp*19 regulates the balance of androgens and estrogens by catalyzing the demethylation of androgen to be oxidated to estrogen [54, 55]. Recently, the P450arom transcripts were found to be significantly different between immotile and motile sperms. Compared with motile sperm fraction from the same sample, a 28–30% decrease of the amount of *P450arom* mRNA is observed in immotile sperms [56]. While for the genes of the protamines PRM1, the opposite was observed. Lambard *et al*. (2004) found the number of *PRM1* transcripts in low-motility sperms was significantly higher than that in high-motility sperms [57]. On the contrary, Ganguly *et al*. (2013) found that the amount of *PRM1* mRNA in normal-motility sperms was significantly higher than in low-motility sperms [58]. It appears that the quantity of *PRM1* transcripts varies according to the sperm motility, and further evidence is needed. Evaluation of endothelial nitric oxide synthase (*eNOS*) gene and neuronal nitric oxide synthase (*nNOS*) gene showed that the two transcripts were undetectable in most of the high-motility sperms, and only detected in low-motility sperm samples [57]. The high levels of *eNOS* and *nNOS* transcripts in low motile sperms may result in the excessive production of NO, which is responsible for the inhibition of sperm motility [59]. Genes of sperm cation channel-like protein family play important roles in different aspects of mammalian sperm functions, such as sperm motility, capacitation and the acrosome reaction [60, 61]. Their transcripts' quantity is different in sperms with different motility. For instance, the transcript level of *CatSper2* and *CatSper3* in high-motility sperms was significantly higher than that of in low-motility sperms [62]. Jing *et al*. revealed a positive correlation between *CatSper1* transcript level and sperm motility [63]. Additionally, Chen *et al*. unveiled that the number of expressed nuclear factor erythroid 2-related factor 2 (*NFR2*) gene in low-motility sperms was significantly lower than in high-motility sperms [64].

The transcript number of genes in sperm appears to vary with different motilities, attributing to an increase of the transcriptional activity, a decrease at the translational level or a longer half-life of the RNAs [56, 65]. Nevertheless for the same amount of RNA analyzed, the level of specific P450arom transcript was significantly lower in the immotile sperm cells, as also reported for the PAF-receptor mRNA [66]. A recent study showed that the transcript quantity of the mitochondrial NADH dehydrogenase 2 (*MT-ND2*) gene in asthenospermic sperms was significantly lower than in normal-motility

sperms, as was the transcript number of three genes annexin A2 (*ANXA2*), bromodomain containing 2 (*BRD2*), and ornithine decarboxylase antizyme 3 (*OAZ35*). Among them, the transcripts of *ANXA2* and *BRD2* were positively correlated with sperm motility [67]. The quantity of transcripts is different in sperms with different motility, and this difference leads to a series of discussion questioning the presence of sperm transcriptional activity. Detection of low level transcription in sperms, especially under certain conditions such as capacitation, and acrosome reaction, has been documented [52, 68]. Further verification is needed to support the idea.

The transcripts in sperm vary with capacitation

Unless they undergo capacitation, mammalian epididymal and ejaculated sperms do not have the ability to fertilize the oocyte *in vitro* [69, 70]. It has been confirmed that sperm proteins change after capacitation [21, 71]. Lambard *et al.* (2004) found that protamine transcripts did not significantly change, but the *c-myc* transcripts partially or completely disappeared in the sperm of healthy humans four hours after capacitation. Lee *et al.* (2011) analyzed the transcripts of *Myc*, *CYP19A1* encoding aromatase, domain-containing protein 2 (*ADAM2*), *PRM1* and *PRM2* in pig sperms before and after capacitation by RT-PCR and quantitative real-time PCR. Their results showed that the transcriptional level of *PRM1* and *PRM2* did not significantly change, but *MYC*, *CYP19A1*, and *ADAM2* was significantly down-regulated after capacitation [72]. The decrease of some transcripts after capacitation might result from the increase of the translational activities during capacitation for more protein synthesis [54, 73]. Transcriptional activities in the head and midpiece regions of sperm during capacitation had been detected, although the studies on transcript increase had not yet been reported [68]. It needs to be further verified whether the transcriptional activity increase or not after capacitation.

The transcripts in sperm vary with cryopreservation

Semen cryopreservation promotes the application of artificial insemination (AI) in livestock breeding, and draws more attention to the impacts of cryopreservation on sperm transcripts. Ostermeier *et al.* (2005) tested the expression of the expressed sequence tags (ESTs) from human sperm samples exposed to different freezing-thawing cycles [74]. The authors found that the number of ESTs in fresh semen was highest and there were 59 more ESTs in sperms treated with one *vs* three freezing-thawing cycles. Garcia-Herrero et al. detected the transcripts in fresh and frozen sperms used for intracytoplasmic sperm injection (ICSI) and analyzed the differential expression between sperms that resulted in pregnancy and those that didn't (2011). Transcripts of 19,229 genes were detected in fresh semen, while 18,095 were found in frozen semen. The transcript difference was also found in fresh sperms between pregnancy and nonpregnancy groups, while no difference was detected in frozen spermatozoa between pregnancy and nonpregnancy groups [75]. In addition, Valcarce *et al.* (2013) found that the transcript number in sperms after freezing treatment was significantly reduced [76]. Therefore, frozen treatment significantly decreased the number of transcripts in sperms, and the more frequent the freezing-thawing treatments, the fewer the number of transcripts. Among these transcriptional variations, different trends for different genes were observed. The transcript number of an RNA-binding protein gene *CIRBP* in bovine frozen sperm was reduced, while the transcript levels of genes encoding cold shock protein A (CspA), heat shock protein 60 (HSP60), and heat shock protein 10 (HSP10) were increased after freezing and thawing [77]. These two trends were also detected in Chen's study (2015). Transcripts of 16 genes were significantly increased and transcripts of 3 other genes were significantly reduced after cryopreservation. The up-regulation of *PRKCE* and unknown gene *R1G7* after cryopreservation may be related to anti-oxidation and strengthening of the acrosome reaction [78]. Recently, a study on boar spermatozoa found that the expression level of 3 microRNA in cryopreserved spermatozoa are higher than in fresh ejaculate [79]. This variation of transcript quantity between frozen and fresh sperm may be induced by the freezing-thawing treatment, which maybe affect mRNA–protein interaction and make mRNA more susceptible to degradation [76]. The increase of transcript may be due to the freezing stress which led to the transcriptional activity increasing [80].

Discussion

Sperm constitutes the only vector that can convey and perpetuate life for male. As a single haploid cell, sperms perform all of their vital processes within the female reproductive tract, including capacitation, movement, and recognition and binding with the ovum.

Sperm can adapt to the external environment so as to complete this series of crucial events requisite for life, and a series of transcriptional and translational events may thereby play an important role. Transcripts vary with spermiogenesis and capacitation state, and they also vary with differential sperm motilities, the freezing-thawing cycle (Table 1), and different sex chromosomes the sperm carries [80]. However, chromatin structure is compacted in sperms, it deserves further study to investigate whether it has the potential for transcription to successfully regulate the viability of this single reproductive cell.

There are different RNA populations in mature sperms from fertile and infertile men, and some highly associated with sperm motility, capacitation, and other parameters [57, 59]. Another important point is that some sperm RNAs are present in zygotes and early embryos, and regulate epigenetic events affecting embryonic development or function as a regulator participating in cell signaling processes during development of the zygote and embryo [81]. Thus, the study of sperm transcript may have profound clinical implications in the diagnosis of male infertility as well as in the practice of assisted reproductive technologies. Are the sperm RNAs which play important roles the remnant of spermatogenesis or the result of sperm transcription? It is not clear at present and needs to be further studied.

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Table 1. Different opinions about transcriptional activity in mature sperm

Opinions	References	Species	Method
Post-meiotic transcription	Welch *et al.,* 2006	Rat	Northern blotting
	Barreau *et al.,* 2008	Drosophila	Situ hybridization
The transcripts in sperm vary with different sperm motilities	Lambard *et al.,* 2003	Human	RT-PCR
	Lambard *et al.,* 2004	Human	RT-PCR
	Li *et al.,* 2007	Mouse and human	RT-PCR
	Jodar *et al.,* 2012	Human	qPCR
	Chen *et al.,* 2012	Human	qPCR
	Ganguly *et al.,* 2013	Bull	qPCR
The transcripts in sperm vary with capacitation	Lambard *et al.,* 2004	Human	RT-PCR
	Lee *et al.,* 2011	Human	Microarrays
The transcripts in sperm vary with cryopreservation	Ostermeier *et al.,* 2005	Human	Microarray
	García-Herrero *et al.,* 2011	Human	Microarray
	Valcarce *et al.,* 2013	Human	qPCR
	Chen *et al.,* 2015	Bull	Microarray

References

1. **Rathke C, Baarends WM, Awe S, Renkawitz-Pohl R.** Chromatin dynamics during spermiogenesis. *Biochim Biophys Acta* 2014; **1839**: 155–168. [Medline] [CrossRef]
2. **Gaucher J, Reynoird N, Montellier E, Boussouar F, Rousseaux S, Khochbin S.** From meiosis to postmeiotic events: the secrets of histone disappearance. *FEBS J* 2010; **277**: 599–604. [Medline] [CrossRef]
3. **Gaucher J, Boussouar F, Montellier E, Curtet S, Buchou T, Bertrand S, Hery P, Jounier S, Depaux A, Vitte AL, Guardiola P, Pernet K, Debernardi A, Lopez F, Holota H, Imbert J, Wolgemuth DJ, Gérard M, Rousseaux S, Khochbin S.** Bromodomain-dependent stage-specific male genome programming by Brdt. *EMBO J* 2012; **31**: 3809–3820. [Medline] [CrossRef]
4. **Goudarzi A, Shiota H, Rousseaux S, Khochbin S.** Genome-scale acetylation-dependent histone eviction during spermatogenesis. *J Mol Biol* 2014; **426**: 3342–3349. [Medline] [CrossRef]
5. **Yang Z, Gallicano GI, Yu QC, Fuchs E.** An unexpected localization of basonuclin in the centrosome, mitochondria, and acrosome of developing spermatids. *J Cell Biol* 1997; **137**: 657–669. [Medline] [CrossRef]
6. **Steger K.** Transcriptional and translational regulation of gene expression in haploid spermatids. *Anat Embryol (Berl)* 1999; **199**: 471–487. [Medline] [CrossRef]
7. **Oliva R.** Protamines and male infertility. *Hum Reprod Update* 2006; **12**: 417–435. [Medline] [CrossRef]
8. **Miller D, Ostermeier GC, Krawetz SA.** The controversy, potential and roles of spermatozoal RNA. *Trends Mol Med* 2005; **11**: 156–163. [Medline] [CrossRef]
9. **Hecht NB.** Regulation of haploid expressed genes in male germ cells. *J Reprod Fertil* 1990; **88**: 679–693. [Medline] [CrossRef]
10. **Hecht NB.** Molecular mechanisms of male germ cell differentiation. *BioEssays* 1998; **20**: 555–561. [Medline] [CrossRef]
11. **Fischer BE, Wasbrough E, Meadows LA, Randlet O, Dorus S, Karr TL, Russell S.** Conserved properties of Drosophila and human spermatozoal mRNA repertoires. *Proc Biol Sci* 2012; **279**: 2636–2644. [Medline] [CrossRef]
12. **Braun RE.** Packaging paternal chromosomes with protamine. *Nat Genet* 2001; **28**: 10–12. [Medline] [CrossRef]
13. **Luk AC, Chan WY, Rennert OM, Lee TL.** Long noncoding RNAs in spermatogenesis: insights from recent high-throughput transcriptome studies. *Reproduction* 2014; **147**: R131–R141. [Medline] [CrossRef]
14. **Wu TF, Chu DS.** Sperm chromatin: fertile grounds for proteomic discovery of clinical tools. *Mol Cell Proteomics* 2008; **7**: 1876–1886. [Medline] [CrossRef]
15. **Brewer LR, Corzett M, Balhorn R.** Protamine-induced condensation and decondensation of the same DNA molecule. *Science* 1999; **286**: 120–123. [Medline] [CrossRef]
16. **D'Occhio MJ, Hengstberger KJ, Johnston SD.** Biology of sperm chromatin structure and relationship to male fertility and embryonic survival. *Anim Reprod Sci* 2007; **101**: 1–17. [Medline] [CrossRef]
17. **Miller D, Brinkworth M, Iles D.** Paternal DNA packaging in spermatozoa: more than the sum of its parts? DNA, histones, protamines and epigenetics. *Reproduction* 2010; **139**: 287–301. [Medline] [CrossRef]
18. **Rathke C, Baarends WM, Jayaramaiah-Raja S, Bartkuhn M, Renkawitz R, Renkawitz-Pohl R.** Transition from a nucleosome-based to a protamine-based chromatin configuration during spermiogenesis in Drosophila. *J Cell Sci* 2007; **120**: 1689–1700. [Medline] [CrossRef]
19. **Kierszenbaum AL, Tres LL.** Structural and transcriptional features of the mouse spermatid genome. *J Cell Biol* 1975; **65**: 258–270. [Medline] [CrossRef]
20. **Zhao Y, Li Q, Yao C, Wang Z, Zhou Y, Wang Y, Liu L, Wang Y, Wang L, Qiao Z.** Characterization and quantification of mRNA transcripts in ejaculated spermatozoa of fertile men by serial analysis of gene expression. *Hum Reprod* 2006; **21**: 1583–1590. [Medline] [CrossRef]
21. **Boerke A, Dieleman SJ, Gadella BM.** A possible role for sperm RNA in early embryo development. *Theriogenology* 2007; **68**(Suppl 1): S147–S155. [Medline] [CrossRef]
22. **Dadoune JP.** Spermatozoal RNAs: what about their functions? *Microsc Res Tech* 2009; **72**: 536–551. [Medline] [CrossRef]
23. **Bourchis D, Voinnet O.** A small-RNA perspective on gametogenesis, fertilization, and early zygotic development. *Science* 2010; **330**: 617–622. [Medline] [CrossRef]
24. **Goodwin LO, Karabinus DS, Pergolizzi RG.** Presence of N-cadherin transcripts in mature spermatozoa. *Mol Hum Reprod* 2000; **6**: 487–497. [Medline] [CrossRef]
25. **Goodwin LO, Karabinus DS, Pergolizzi RG, Benoff S.** L-type voltage-dependent calcium channel α-1C subunit mRNA is present in ejaculated human spermatozoa. *Mol Hum Reprod* 2000; **6**: 127–136. [Medline] [CrossRef]
26. **Grivna ST, Beyret E, Wang Z, Lin H.** A novel class of small RNAs in mouse spermatogenic cells. *Genes Dev* 2006; **20**: 1709–1714. [Medline] [CrossRef]
27. **Hosken DJ, Hodgson DJ.** Why do sperm carry RNA? Relatedness, conflict, and control. *Trends Ecol Evol* 2014; **29**: 451–455. [Medline] [CrossRef]
28. **Kumar G, Patel D, Naz RK.** c-MYC mRNA is present in human sperm cells. *Cell Mol Biol Res* 1993; **39**: 111–117. [Medline]
29. **Pessot CA, Brito M, Figueroa J, Concha II, Yañez A, Burzio LO.** Presence of RNA in the sperm nucleus. *Biochem Biophys Res Commun* 1989; **158**: 272–278. [Medline] [CrossRef]
30. **Richter W, Dettmer D, Glander H.** Detection of mRNA transcripts of cyclic nucleotide phosphodiesterase subtypes in ejaculated human spermatozoa. *Mol Hum Reprod* 1999; **5**: 732–736. [Medline] [CrossRef]
31. **Feugang JM, Rodriguez-Osorio N, Kaya A, Wang H, Page G, Ostermeier GC, Topper EK, Memili E.** Transcriptome analysis of bull spermatozoa: implications for male fertility. *Reprod Biomed Online* 2010; **21**: 312–324. [Medline] [CrossRef]
32. **Chalmel F, Rolland AD, Niederhauser-Wiederkehr C, Chung SS, Demougin P, Gattiker A, Moore J, Patard JJ, Wolgemuth DJ, Jégou B, Primig M.** The conserved transcriptome in human and rodent male gametogenesis. *Proc Natl Acad Sci USA* 2007; **104**: 8346–8351. [Medline] [CrossRef]
33. **Grunewald S, Paasch U, Glander HJ, Anderegg U.** Mature human spermatozoa do not transcribe novel RNA. *Andrologia* 2005; **37**: 69–71. [Medline] [CrossRef]
34. **Schäfer M, Nayernia K, Engel W, Schäfer U.** Translational control in spermatogenesis. *Dev Biol* 1995; **172**: 344–352. [Medline] [CrossRef]
35. **Wykes SM, Krawetz SA.** The structural organization of sperm chromatin. *J Biol Chem* 2003; **278**: 29471–29477. [Medline] [CrossRef]

36. **Balhorn R, Gledhill BL, Wyrobek AJ.** Mouse sperm chromatin proteins: quantitative isolation and partial characterization. *Biochemistry* 1977; **16**: 4074–4080. [Medline] [CrossRef]
37. **Gatewood JM, Cook GR, Balhorn R, Schmid CW, Bradbury EM.** Isolation of four core histones from human sperm chromatin representing a minor subset of somatic histones. *J Biol Chem* 1990; **265**: 20662–20666. [Medline]
38. **Soon LL, Ausio J, Breed WG, Power JH, Muller S.** Isolation of histones and related chromatin structures from spermatozoa nuclei of a dasyurid marsupial, Sminthopsis crassicaudata. *J Exp Zool* 1997; **278**: 322–332. [Medline] [CrossRef]
39. **Ward WS.** Function of sperm chromatin structural elements in fertilization and development. *Mol Hum Reprod* 2010; **16**: 30–36. [Medline] [CrossRef]
40. **Kramer JA, McCarrey JR, Djakiew D, Krawetz SA.** Human spermatogenesis as a model to examine gene potentiation. *Mol Reprod Dev* 2000; **56**(Suppl): 254–258. [Medline] [CrossRef]
41. **Oliva R, Mezquita C.** Marked differences in the ability of distinct protamines to disassemble nucleosomal core particles in vitro. *Biochemistry* 1986; **25**: 6508–6511. [Medline] [CrossRef]
42. **Oliva R, Bazett-Jones D, Mezquita C, Dixon GH.** Factors affecting nucleosome disassembly by protamines in vitro. Histone hyperacetylation and chromatin structure, time dependence, and the size of the sperm nuclear proteins. *J Biol Chem* 1987; **262**: 17016–17025. [Medline]
43. **Giordano R, Magnano AR, Zaccagnini G, Pittoggi C, Moscufo N, Lorenzini R, Spadafora C.** Reverse transcriptase activity in mature spermatozoa of mouse. *J Cell Biol* 2000; **148**: 1107–1113. [Medline] [CrossRef]
44. **Gilbert I, Bissonnette N, Boissonneault G, Vallée M, Robert C.** A molecular analysis of the population of mRNA in bovine spermatozoa. *Reproduction* 2007; **133**: 1073–1086. [Medline] [CrossRef]
45. **Esakky P, Hansen DA, Drury AM, Moley KH.** Molecular analysis of cell type-specific gene expression profile during mouse spermatogenesis by laser microdissection and qRT-PCR. *Reprod Sci* 2013; **20**: 238–252. [Medline] [CrossRef]
46. **Catlin NR, Huse SM, Boekelheide K.** The stage-specific testicular germ cell apoptotic response to low-dose X-irradiation and 2,5-hexanedione combined exposure. I: Validation of the laser capture microdissection method for qRT-PCR array application. *Toxicol Pathol* 2014; **42**: 1221–1228. [Medline] [CrossRef]
47. **Dadoune JP.** The cellular biology of mammalian spermatids: a review. *Bull Assoc Anat (Nancy)* 1994; **78**: 33–40. [Medline]
48. **Dadoune JP.** The nuclear status of human sperm cells. *Micron* 1995; **26**: 323–345. [Medline] [CrossRef]
49. **Dadoune JP, Siffroi JP, Alfonsi MF.** Transcription in haploid male germ cells. *Int Rev Cytol* 2004; **237**: 1–56. [Medline] [CrossRef]
50. **Barreau C, Benson E, Gudmannsdottir E, Newton F, White-Cooper H.** Post-meiotic transcription in Drosophila testes. *Development* 2008; **135**: 1897–1902. [Medline] [CrossRef]
51. **Miteva K, Valkov N, Goncharova-Peinova J, Kovachev K, Zlatarev S, Pironcheva G, Russev G.** Electron microscopic demonstration of transcription of ram sperm chromatin. *Microbios* 1995; **84**: 91–96. [Medline]
52. **Welch JE, Barbee RR, Magyar PL, Bunch DO, O'Brien DA.** Expression of the spermatogenic cell-specific glyceraldehyde 3-phosphate dehydrogenase (GAPDS) in rat testis. *Mol Reprod Dev* 2006; **73**: 1052–1060. [Medline] [CrossRef]
53. **Vibranovski MD, Chalopin DS, Lopes HF, Long M, Karr TL.** Direct evidence for postmeiotic transcription during Drosophila melanogaster spermatogenesis. *Genetics* 2010; **186**: 431–433. [Medline] [CrossRef]
54. **Carreau S, Lambard S, Delalande C, Denis-Galeraud I, Bilinska B, Bourguiba S.** Aromatase expression and role of estrogens in male gonad : a review. *Reprod Biol Endocrinol* 2003; **1**: 35. [Medline] [CrossRef]
55. **Saez JM.** Leydig cells: endocrine, paracrine, and autocrine regulation. *Endocr Rev* 1994; **15**: 574–626. [Medline] [CrossRef]
56. **Lambard S, Galeraud-Denis I, Bouraïma H, Bourguiba S, Chocat A, Carreau S.** Expression of aromatase in human ejaculated spermatozoa: a putative marker of motility. *Mol Hum Reprod* 2003; **9**: 117–124. [Medline] [CrossRef]
57. **Lambard S, Galeraud-Denis I, Martin G, Levy R, Chocat A, Carreau S.** Analysis and significance of mRNA in human ejaculated sperm from normozoospermic donors: relationship to sperm motility and capacitation. *Mol Hum Reprod* 2004; **10**: 535–541. [Medline] [CrossRef]
58. **Ganguly I, Gaur GK, Kumar S, Mandal DK, Kumar M, Singh U, Kumar S, Sharma A.** Differential expression of protamine 1 and 2 genes in mature spermatozoa of normal and motility impaired semen producing crossbred Frieswal (HF×Sahiwal) bulls. *Res Vet Sci* 2013; **94**: 256–262. [Medline] [CrossRef]
59. **Rosselli M, Dubey RK, Imthurn B, Macas E, Keller PJ.** Effects of nitric oxide on human spermatozoa: evidence that nitric oxide decreases sperm motility and induces sperm toxicity. *Hum Reprod* 1995; **10**: 1786–1790. [Medline] [CrossRef]
60. **Darszon A, Hernández-Cruz A.** T-type Ca2+ channels in spermatogenic cells and sperm. *Pflugers Arch* 2014; **466**: 819–831. [Medline] [CrossRef]
61. **Jimenez-Gonzalez C, Michelangeli F, Harper CV, Barratt CL, Publicover SJ.** Calcium signalling in human spermatozoa: a specialized toolkit of channels, transporters and stores. *Hum Reprod Update* 2006; **12**: 253–267. [Medline] [CrossRef]
62. **Li HG, Ding XF, Liao AH, Kong XB, Xiong CL.** Expression of CatSper family transcripts in the mouse testis during post-natal development and human ejaculated spermatozoa: relationship to sperm motility. *Mol Hum Reprod* 2007; **13**: 299–306. [Medline] [CrossRef]
63. **Jing J, Fu H, Lin C.** Expression of CatSper1 mRNA in human mature spermatozoa and its relationship with sperm motility. *Chinese J Clin Lab Sci* 2012; **3**: 027.
64. **Chen K, Mai Z, Zhou Y, Gao X, Yu B.** Low NRF2 mRNA expression in spermatozoa from men with low sperm motility. *Tohoku J Exp Med* 2012; **228**: 259–266. [Medline] [CrossRef]
65. **Genissel C, Levallet J, Carreau S.** Regulation of cytochrome P450 aromatase gene expression in adult rat Leydig cells: comparison with estradiol production. *J Endocrinol* 2001; **168**: 95–105. [Medline] [CrossRef]
66. **Roudebush WE, Wild MD, Maguire EH.** Expression of the platelet-activating factor receptor in human spermatozoa: differences in messenger ribonucleic acid content and protein distribution between normal and abnormal spermatozoa. *Fertil Steril* 2000; **73**: 967–971. [Medline] [CrossRef]
67. **Jodar M, Kalko S, Castillo J, Ballescà JL, Oliva R.** Differential RNAs in the sperm cells of asthenozoospermic patients. *Hum Reprod* 2012; **27**: 1431–1438. [Medline] [CrossRef]
68. **Naz RK.** Effect of actinomycin D and cycloheximide on human sperm function. *Arch Androl* 1998; **41**: 135–142. [Medline] [CrossRef]
69. **Chang MC.** Fertilizing capacity of spermatozoa deposited into the fallopian tubes. *Nature* 1951; **168**: 697–698. [Medline] [CrossRef]
70. **Florman HM, First NL.** The regulation of acrosomal exocytosis. I. Sperm capacitation is required for the induction of acrosome reactions by the bovine zona pellucida in vitro. *Dev Biol* 1988; **128**: 453–463. [Medline] [CrossRef]
71. **Gur Y, Breitbart H.** Mammalian sperm translate nuclear-encoded proteins by mitochondrial-type ribosomes. *Genes Dev* 2006; **20**: 411–416. [Medline] [CrossRef]
72. **Lee CK, Hwang JY, Mulligan BP, Kim HM, Ka HH.** Analysis of messenger RNA level changes for selected genes during capacitation in ejaculated boar spermatozoa. *Biol Reprod* 2011; **85**: 806. [CrossRef]
73. **Siffroi JP, Dadoune JP.** Accumulation of transcripts in the mature human sperm nucleus: implication of the haploid genome in a functional role. *Ital J Anat Embryol* 2001; **106**(Suppl 2): 189–197. [Medline]
74. **Ostermeier GC, Goodrich RJ, Diamond MP, Dix DJ, Krawetz SA.** Toward using stable spermatozoal RNAs for prognostic assessment of male factor fertility. *Fertil Steril* 2005; **83**: 1687–1694. [Medline] [CrossRef]
75. **García-Herrero S, Garrido N, Martínez-Conejero JA, Remohí J, Pellicer A, Meseguer M.** Differential transcriptomic profile in spermatozoa achieving pregnancy or not via ICSI. *Reprod Biomed Online* 2011; **22**: 25–36. [Medline] [CrossRef]
76. **Valcarce DG, Cartón-García F, Herráez MP, Robles V.** Effect of cryopreservation on human sperm messenger RNAs crucial for fertilization and early embryo development. *Cryobiology* 2013; **67**: 84–90. [Medline] [CrossRef]
77. **Wang H.** The research of the relationship about the express of related protein/gene and bull sperm vitality after freezing. Northwest Agriculture and Forestry University, 2012.
78. **Chen X, Wang Y, Zhu H, Hao H, Zhao X, Qin T, Wang D.** Comparative transcript profiling of gene expression of fresh and frozen-thawed bull sperm. *Theriogenology* 2015; **83**: 504–511. [Medline] [CrossRef]
79. **Zhang Y, Zeng CJ, He L, Ding L, Tang KY, Peng WP.** Selection of endogenous reference microRNA genes for quantitative reverse transcription polymerase chain reaction studies of boar spermatozoa cryopreservation. *Theriogenology* 2015; **83**: 634–641. [Medline] [CrossRef]
80. **Chen X, Yue Y, He Y, Zhu H, Hao H, Zhao X, Qin T, Wang D.** Identification and characterization of genes differentially expressed in X and Y sperm using suppression subtractive hybridization and cDNA microarray. *Mol Reprod Dev* 2014; **81**: 908–917. [Medline] [CrossRef]
81. **Krawetz SA.** Paternal contribution: new insights and future challenges. *Nat Rev Genet* 2005; **6**: 633–642. [Medline] [CrossRef]