Non-Invasive Approaches to Epigenetic-Based Sperm Selection

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Since sperm size and form do not necessarily provide information on internal sperm structures, novel sperm markers need to be found in order to conduct assisted reproductive therapies (ART) successfully. Currently, the priority of andrologists is not only to select those sperm able to fertilize the oocyte, but also a high quality of sperm that will guarantee a healthy embryo. Evidence of this shows us the importance of studying sperm intensively on genetic and epigenetic levels, because these could probably be the cause of a percentage of infertility diagnosed as idiopathic. Thus, more attention is being paid to posttranslational modifications as the key for better understanding of the fertilization process and its impact on embryo and offspring. Advances in the discovery of new sperm markers should go hand in hand with finding appropriate techniques for selecting the healthiest sperm, guaranteeing its non-invasiveness. To date, most sperm selection techniques can be harmful to sperm due to centrifugation or staining procedures. Some methods, such as microfluidic techniques, sperm nanopurifications, and Raman spectroscopy, have the potential to make selection gentle to sperm, tracking small abnormalities undetected by methods currently used. The fact that live cells could be analyzed without harmful effects creates the expectation of using them routinely in ART. In this review, we focus on the combination of sperm epigenetic status (modifications) as quality markers, with non-invasive sperm selection methods as novel approaches to improve ART outcomes.

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Background

Widespread Assisted Reproductive Therapies (ART), such as in vitro fertilization (IVF) and mainly intracytoplasmic sperm injection (ICSI), have opened up a new perspective on infertility treatment. The successful outcomes of ART are based on selecting the most suitable gametes with which to conduct the process. And, despite the selection of oocytes in the female being limited due to the small number of oocytes with each individual feature, in the case of males, it is possible to perform strict selection to choose the finest spermatozoa with which to improve the outcome of ART.

Sperm heterogeneity in ejaculate is widely recognized. This diversity can be found at different levels and characteristics such as shape, motility, DNA content, or membrane composition [1,2]. Recently it has been shown that sperm are also epigenetically heterogeneous, although few reports have addressed this approach [3–5]. Epigenetic alterations of the genome and associated posttranslational modifications of DNA-binding histones equally affect gamete development, maturation, and embryogenesis. Therefore, posttranslational modification (methylation, acetylation, phosphorylation, ubiquitination, and SUMOylation) and noncoding RNAs have been revealing such key markers of fertilization and embryo development abilities in sperm [6–9]. Their evaluation is paramount to provide relevant information for ART. Furthermore, the study of sperm epigenetic markers is also crucial because it has been suggested that they are involved in the etiology of male idiopathic infertility [10].

On the other hand, it might be advisable to follow up sperm changes derived from the use of ART and its consequences. The application of ART includes stages outside the male and female reproductive tracts, during which spermatozoa are subjected to procedures aimed at maximizing reproductive success [11]. This in vitro environment can affect sperm characteristics and function. It is currently also known that epigenetic changes are more frequent than DNA mutations, and their contributions during the performance of human ART is not well defined. Although many researchers have reported a higher incidence of imprinting disorders in ART offspring [12–14], a clear relationship has not been demonstrated. Recently, it has been reported that epigenetic alterations could rather arise from the process of gametogenesis rather than ART itself [15,16]. Thus, it can therefore be detected in the semen of the father. However, we should not overlook the effect of our manipulation with gametes on the stability of the epigenetic process. Based on these facts, sperm handling should be sensitive and invasiveness should be the last resort.

As mentioned above, the selection of viable sperm is an essential procedure to carry out ART. Thus far, centrifugation over discontinuous density gradient (DG) has remained the most popular method, together with swim-up [17–21]. Both methods select morphologically normal and motile sperm, and, although these spermatozoa are expected to be epigenetically stable, few studies have been carried out in this direction [22]. Furthermore, there is evidence to support the fact that sperm morphology does not always reflect DNA status [23,24]. It is therefore crucial to find alternative sperm selection methods which allow us to isolate sperm based on novel parameters to better monitor sperm function.

For detection of distinct epigenetic markers, staining of the cell is usually required, together with flow cytometry or immunocytochemistry [25]. These approaches are valid for identifying mistakes in posttranslational modifications, but the sperm cannot subsequently be used in ART. Estimation of markers based on the epigenome for evaluation of sperm quality, in combination with harmless procedures that are able to detect them, could be one of the solutions to help infertile couples. In this context, non-invasive and label-free methods, such as microfluidics, nanopurification, and Raman spectroscopy, are promising.

Microfluidics

The female reproductive tract has many functions, including the facilitation of sperm migration to the site of fertilization, as well as the selection of healthy sperm [26]. The mechanisms by which the female genital tract selects spermatozoa still remain unknown, but it selects against spermatozoa containing damaged DNA, acting as a biological sensor to screen spermatozoa. This fact demonstrates the existence of unrecognized processes for detection and interpretation of markers on the sperm surface that link phenotypic and genotypic qualities of each individual sperm [27]. It has also been reported that the oviduct responds to sperm presence by modifying the oviductal environment [28].

It is known that many of these characteristics of the oviductal environment are impossible to reproduce during ART. However, some features, such as unidirectional, laminar or gradient flow, containment in a 3-D physical environment, and changing the chemical composition in the medium, can be obtained in a microfluidic environment [29].

These microfluidic devices use microchannels to sort sperm according to normal morphology, motility, and higher DNA integrity for IVF techniques [30,31]. Important advantages have been shown compared to traditional selection techniques, such as the potential to work with small sperm sample volumes, short processing times, and the ability to manipulate single cells in a non-invasive manner. In addition, the potential to be a versatile tool for selection applications or fundamental.
studies on sperm has also been shown [24,32]. Furthermore, the yield of the selected sperm by microfluidic technique was estimated at 41%, what is comparable to the recovery rate of currently used conventional methods [33].

Another of the advantages of using a microfluidic device in ART is its one-step sorting protocol without the need to centrifuge. Eliminating the centrifugation step minimizes the exposure of sperm to reactive oxygen species (ROS), preserving the integrity of the chromatin [34]. DNA fragmentation is significantly decreased in treated sperm with the microfluidic sperm sorting system [35,36]. Wang et al. [37] compared the swim-up method with a microfluidic device, resulting in a significantly lower rate of DNA damage (16.4% swim-up vs. 8.4% microfluidic). A recent microfluidic study [30] utilized polycarbonate filter paper with different-sized pores to determine how the different pore sizes affect sperm retrieval, ROS production, and DNA integrity. The device was efficient in selecting motile and morphologically normal sperm. In addition, there was significantly less ROS production and improved sperm retrieval using the microfluidics versus the swim-up method [30]. Another research group created a device that uses a radial array of microchannels to select the most motile sperm, and they were able to obtain 80% improvement in sperm DNA integrity after sorting [38].

Decreasing levels of DNA fragmentation and ROS can prevent epigenetic changes, since it has been observed that, in sperm damaged by oxidative stress, impaired DNA sequence prevents the process of DNA methylation [39]. Other studies have suggested that oxidative stress due to smoking affects protamine protein levels and histone retention rates in mature sperm [40,41]. The fact that the epigenetically aberrant sperm populations were preferentially found in men with severe sperm abnormalities and that non-imprinted genes are also affected, suggests that there might be a link between phenotype and epigenotype [4]. However, sometimes normal morphology is not necessarily associated with DNA integrity [24]. Moreover, epigenetic markers had significant variation between samples from different men, as well as significant variation within the same semen sample. This reinforces that epigenetic patterns may be used as biomarkers for sperm quality [42].

Future studies should investigate creating sperm-sorting devices that can isolate spermatozoa to limit genetic diseases, while also maintaining sperm viability. New sperm sorting techniques have been shown to be able to improve DNA integrity, morphology, and motility, but there are still some conflicts as to whether these are significant improvements over the conventional centrifuged-based techniques [43].

In summary, microfluidic technology could offer a new non-invasive method for selecting sperm with good molecular characteristics to prepare sperm for IVF or ICSI, which would greatly improve the current point-of-care ART. Although newer microfluidic-based sperm sorting methods showed promising results, these devices should be thoroughly analyzed for their clinical utility to continue progress in the fields of microfluidics and andrology.

### Sperm Nanopurification

Nanotechnologies and their application have undergone huge expansion in different disciplines, including the medical field. The suggestion exists for the use of nanoparticles in drug delivery, diagnostics, and, in the case of sperm, as a non-invasive sperm selection method [44,45]. While methods such as Raman spectroscopy, described below, can function with any sperm molecules, nanoparticles for sperm selection interact with external molecules of the sperm membrane or acrosome. This could seem to be a disadvantage, but as mentioned before, we are witness to sperm ability reflecting their inner condition on the sperm surface [46,47].

Nanotechnologies function with different nanoparticle materials, but some harmful side effects can be found [48,49]. To date, the most successful method for sperm selection using nanoparticles is magnetic-activated cell sorting (MACS), which separates apoptotic and non-apoptotic spermatozoa [50,51]. However, in recent years, a new method based on magnetic nanoseparation of abnormal sperm has been implemented and was applied in cattle, utilizing ferritin nanoparticles coated with specific antibodies [52]. The sperm nanopurification process is relatively easy to implement. First, a mixture of sperm with ferritin nanoparticles is created and coated with antibodies against specific molecules, especially those which are expressed on the surface of defective sperm [52]. Spermatozoa with these molecules interact with the coated nanoparticles, and a magnetic separator is placed at the bottom of a tube for final nanopurification. Thus, the pellet containing unhealthy sperm attached to nanoparticles is removed. Utilizing this technique for bull semen purification, 25–30% spermatozoa were eliminated and it was possible to obtain double the number of artificial insemination doses per semen collection [47,52]. Success in sperm nanopurification in cattle has been reported, increasing the number of pregnancies after insemination and IVF [52].

As the most suitable antibodies, those against the ligand of lectins PNA (peanut agglutinin from *Arachis hypogaea*) and PSA (*Pisum sativum agglutinin*) that are more abundant on the acrosome membrane of defective sperm [46,53] were selected, as well as ubiquitin, which is a small chaperone molecule known mainly from posttranslational modifications called ubiquitination. While monoubiquitination participates mainly in the regulation of gene transcription, cell signalling, and silencing of X chromosomes [54,55], polyubiquitination plays a...
role in protein turnover by the ubiquitin proteasome system. Ubiquitin as a quality control marker is secreted by epididymal epithelium to eliminate defective spermatozoa by subsequent phagocytosis [56–58]. Nevertheless, some of the defective spermatozoa tagged by extracellular/cell surface ubiquitination are carried over into the ejaculate. This fact supports the utilization of ubiquitin as an appropriate sperm marker [58,59].

The estimation of ubiquitin for sperm selection was confirmed by sperm ubiquitin-tag immunoassay (SUTI). The intensity of ubiquitination in sperm correlated with the fertilization rate and success of ART, such as ICSI and IVF [59–61]. However, SUTI has been combined with flow cytometry or fluorescence microscope requiring sperm staining, which makes subsequent use of these sperm for fertilization in a harmless way unlikely.

The expectation of the application of sperm nanopurification in human reproductive medicine is based on the current information about its non-invasiveness. To date, no harmful side effects have been noted to be caused by using ferritin nanoparticles or the magnetic field, which is a crucial argument for its use in humans [52]. Furthermore, ubiquitin seems to be the proper marker for sperm selection, not only for its accessibility on the sperm membrane, but mainly for its ability to reflect the DNA status [60]. All this information supports the application of sperm nanopurification in connection with ubiquitin in human reproductive medicine and, due to its non-invasiveness, in the treatment of couples who have problems conceiving.

Raman Spectroscopy

Sperm heterogeneity is widely accepted [4,5], and is based on several sperm features, including slight molecular differences which determine the sperm function. Changes in DNA packaging or epigenetic modifications can be important [62–64]. However, these small but important details are difficult to evaluate by standard methods, without disturbing or even destroying the sperm cell.

Raman spectroscopy (RS) is an optical laser-based technique which provides information on the vibrational energies of the biomolecular constituents of the sample [66]. Any changes in structure are translated into distinct Raman spectra from each molecule or tissue, without use of fixatives or labels [66–68]. However, a laser with suitable wavelengths and intensities to guarantee its invasiveness is required [68]. The combination of Raman spectroscopy with confocal microscopy, called Raman microspectroscopy, allows us to obtain 3D spatial resolution and makes possible investigations into single live cell tracking in situ changes in cell components [68,69].

Although RS has been used in different biomedical fields [70,71], in reproductive medicine and mainly in andrology, it has started to be utilized in recent years [71–73]. Using RS, researchers have characterized different sperm regions such as the head, acrosome, middle piece, and flagellum, as well as inner organelles such as the nucleus and mitochondria [71–73]. This success is a prerequisite for deeper sperm characterization on a molecular level.

Currently, Raman spectroscopy is used mainly in the detection of sperm nuclear damage [67], and also in sex sperm selection [71]. The PO backbone of DNA is characterized by peak intensity from 1055 cm\(^{-1}\) to 1095 cm\(^{-1}\) [67,69,73]. Any changes in the intensity are signs of DNA damage, and it is possible to make a map of sites with DNA fragmentation. The results that were obtained by Raman microspectroscopy in the field of DNA evaluation are in correlation with the DNA fragmentation index (DFI), which is associated with infertility and spontaneous abortions [66,69,74]. For sex sperm selection, variations of Raman peaks from DNA content and sex membrane proteins were reported in bovine sperm [71,75]. These results showed, with more than 90% accuracy, that RS may be applied in the near future for sperm sexing in a label-free and non-invasive manner, compared with the sex sorting by flow cytometry used to date [72,76]. Recently, the damage induced by sperm sorting and freezing-thawing procedures have been quantified by laser tweezers Raman spectroscopy [72]. These authors reported a variation of DNA, lipid, carbohydrates, and protein contents in sperm during flow cytometry process. Liu et al. [77] also showed the ability of Raman microspectroscopy to distinguish zona pellicula-bound sperm from unbound sperm, identifying differences in the intensity of Raman spectra on the acrosome region.

Thus, Raman spectroscopy allows us to evaluate DNA and RNA, as well as histones and protamines [78–80]. Popleineau et al. [78] used laser tweezers Raman spectroscopy to isolate a living human cell and monitor epigenetic modifications. These researchers treated Jurkat cells with histone deacetylase inhibitors, which resulted in an increase of histone code acetylation and changes in chromatin. The aforementioned effect was analyzed by different methods (laser tweezers Raman spectroscopy, electrophoresis, and nuclear image cytometry). Laser tweezers Raman spectroscopy showed the best discrimination between treated and control cells.

Noticeably, according to recent studies, if epigenetic modifications of sperm could be evaluated by non-invasive Raman spectroscopy, similarly to Jurkat cells, they would be suitable biomarkers of sperm quality in routine ART, providing relevant information on male infertility.

Conclusion and Perspectives

Correct sperm evaluation and selection of the most representative markers may be the key to resolving male infertility.
problems. The most feasible option for finding informative biomarkers that will be a guarantee of successful fertilization and proper embryo and offspring development could be found in the epigenome. Many researchers have suggested the possible usefulness to ART of analyzing epigenetic markers in routine sperm analysis [6,11,15,16]. However, further studies are required to confirm the relationship between sperm epigenetic features and the outcome of ART, as well as alterations in offspring generated by ART.

Correct chromatin structure involves the precise realization and timing of posttranslational modifications and protamination, essential for healthy progenitive sperm. Accordingly, deeper insight into DNA structure and epigenome generally seems to be promising in the clarification of processes for sperm fertilization and embryo development [6–8,10,81,82]. Posttranslational processes have an evident impact on embryo imprinting, and defects of imprinting participate in different offspring disorders, such as Angelman, Beckwith-Wiedemann, Prader-Willi, Silver-Russell, Goiter, Kabuki, and Claes-Jensen X-linked mental retardation syndrome [12,13,83,84]. For all these reasons, the development of adequate therapeutic options and sperm selection technologies based on epigenetic quality are crucial for improving ART outcomes [4].

Although the importance of epigenetics is obvious, it is currently not used in sperm selection methods or other ART techniques. The latest knowledge forces us to consider new perspectives for tracking these novel sperm parameters. The above-mentioned techniques (microfluidic device, sperm nanopurification, and Raman spectroscopy) could be options (Figure 1). Furthermore, a combination of microfluidic and Raman spectroscopy is possible and could increase sperm selection efficiency in some infertility cases [24,85].

These methods are not only non-invasive, they also have a huge potential for identification of epigenetic markers and use in successful ART. Actual studies of potential techniques show good results that are in correlation with the DNA fragmentation index (%DFI) and success of fertilization, ICSI, and others [32,36,52]. In addition, another use could be to mimic the natural sperm environment and natural sperm selection in the female genital tract, such as microfluidics [86,87].

However, although the sperm separation methods currently in use cannot use epigenetic markers for selecting undamaged sperm, Raman spectroscopy could be a promising method of identifying small differences in the chemical structure which are associated with changes in cell physiological properties (Figure 1). Furthermore, the most recent information on the application of Raman spectroscopy in evaluation of the histone acetylation state of cancer cells, suggests it could be used in sperm and expanding research to other posttranslational modifications, such as phosphorylation and DNA methylation. The level of histone phosphorylation detected by flow cytometry correlates with DNA damage and infertile male patients [88]. In addition, the DNA methylation pattern varies between fertile normospermic males and IVF patients, even if it seems to be a proper indicator of embryo quality after IVF [89]. However,
it would be better if Raman spectroscopy could be used for these objectives and this seems to be possible.

Progress in Raman spectroscopy suggests that in the near future we will be able to better observe the epigenome of gametes without disturbing or destroying them. The results obtained by these methods show that sperm selected in these ways, in comparison to standard methods, exhibit better quality. Nevertheless, although the results and application in ART seem to be promising, further observations are necessary to confirm their safety in the epigenetic context.

References:

1. Jenkins TG, Aston KI, Trost C et al: Intra-sample heterogeneity of sperm DNA methylation. Mol Hum Reprod, 2015; 21: 313–19
2. Holt VW, Van Look PJ: Concepts in sperm heterogeneity, sperm selection and sperm competition as biological foundations for laboratory tests of semen quality. Reproduction, 2004; 127: 527–35
3. Jenkins TG, Aston KI, Pfueger C et al: Age-associated sperm DNA methylation alterations, possible implications in offspring disease susceptibility. PLoS Genet, 2014; 10: e1004458
4. Laurentino S, Borgmann I, Gromoll J: On the origin of sperm epigenetic heterogeneity. Reproduction, 2016; 151: R71–78
5. Schagdarsurengin U, Steger K: Epigenetics in male reproduction. Fertil Steril, 2012; 97: 1369–704
6. Sharma U, Conine CC, Shea JM et al: Biogenesis and function of tRNA fragments during sperm maturation and fertilization in mammals. Science, 2016; 351: 391–96
7. Sikkena K, Erkse S, Godmann M et al: Disruption of histone methylation in developing sperm impairs offspring health transgenerationally. Science, 2015; 350: aab2006
8. Tang D, Huang Y, Liu W, Zhang X: Up-regulation of microRNA-210 is associated with spermato genesis by targeting IGFB in male infertility. Med Sci Monit, 2016; 12: 2905–10
9. Carrell DT: Sperm preparation, state-of-the-art-physiological aspects and application of advanced sperm preparation methods. Asian J Androl, 2012; 14: 260–69
10. Carrell DT: Epigenetics of the male gamete. Fertil Steril, 2012; 97: 267–74
11. Yu B, Zhou H, Li M et al: Epigenetic alterations in density selected human spermatozoa for assisted reproduction. PLoS One, 2015; 10: e0104558
12. Han JY, Park J, Jang W et al: A twin sibling with Prader-Willi syndrome caused by imprinting methylation errors in ART. Reprod Biomed Online, 2015; 35: aab2006
13. Hiura H, Okae H, Chiba H et al: Imprinting methylation errors in ART. Reprod Biomed Online, 2015; 35: aab2006
14. Kurinczuk JJ, Bhattacharya S: Rare chromosomal, genetic, and epigenetic-related risks associated with infertility treatment. Semin Fetal Neonatal Med, 2014; 19: 95–105
15. Suárez SS: Mammalian sperm interactions with the female reproductive system for separation of mottled sperm. Asian J Androl, 2012; 14: 260–69
16. de Wagenaar B, Berendsen JT, Bomer JG et al: Microfluidics for sperm research. Biomed Rep, 2016; 5: 18–22
17. de Wagenaar B, Berendsen JT, Bomer JG et al: Microfluidics for sperm research. Biomed Rep, 2016; 5: 18–22
18. Sikkema K, Erkse S, Godmann M et al: Disruption of histone methylation in developing sperm impairs offspring health transgenerationally. Science, 2015; 350: aab2006
19. Tang D, Huang Y, Liu W, Zhang X: Up-regulation of microRNA-210 is associated with spermato genesis by targeting IGFB in male infertility. Med Sci Monit, 2016; 12: 2905–10
20. Carrell DT: Sperm preparation, state-of-the-art-physiological aspects and application of advanced sperm preparation methods. Asian J Androl, 2012; 14: 260–69
21. Xue X, Wang WS, Shi JJ et al: Efficacy of swim-up versus density gradient centrifugation in improving sperm deformity rate and DNA fragmentation index in semen samples from teratozoospermic patients. J Assist Reprod Genet, 2014; 31: 1161–66
22. Henkel R: Sperm preparation, state-of-the-art-physiological aspects and application of advanced sperm preparation methods. Asian J Androl, 2012; 14: 260–69
23. Avendaño C, Oehniger S: DNA fragmentation in morphologically normal spermatozoa, how much should we be concerned in the ICSI era? J Androl, 2011; 32: 356–63
24. Samuel R, Badamjav O, Murphy KE et al: Microfluidics: The future of microdissection. Fertil Steril, 2016; 106: 161–70
25. Křeček J, Stivová L, Pagáčová E et al: Post-translational modifications of histones in human sperm. Cell Biochem, 2015; 116: 2195–209
26. Suarez SS: Mammalian sperm interactions with the female reproductive tract. Cell Tissue Res, 2016; 363: 185–94
27. Holt VW, Fazeli A: The oviduct as a complex mediator of mammalian sperm function and selection. Mol Reprod Dev, 2010; 77: 934–43
28. Almíñana C, Caballero J, Heath PR et al: The battle of the sexes starts in the oviduct, modulation of oviductal transcriptome by X and Y-bearing spermatozoa. BMC Genomics, 2014; 15: 293
29. Wheeler MB, Rubessa M: Integration of microfluidics and mammalian IVF. Mol Hum Reprod, 2016 [Epub ahead of print]
30. Asghar W, Velasco V, Kingsley IL et al: Selection of functional human sperm with higher DNA integrity and fewer reactive oxygen species. Adv Healthc Mater, 2014; 3: 1671–79
31. Knowlton SM, Sadasivam M, Tasoglu S: Microfluidics for sperm research. Trends Biotechnol, 2015; 33: 221–29
32. de Wagenaar B, Berendsen JT, Bomer JG et al: Microfluidic single sperm entrainment and analysis. Lab Chip, 2015; 15: 1294–301
33. Cho BS, Schuster TG, Zhu X et al: Passively driven integrated microfluidic system for separation of motile sperm. Anal Chem, 2003; 75: 1671–75
34. Taken K, Alp HH, Eryilmaz R et al: Oxidative DNA damage to sperm cells and peripheral blood leukocytes in infertile men. Med Sci Monit, 2016; 22: 4289–96
35. Matsuraka K, Uozumi T, Furuichi T et al: Microfluidic device to reduce treatment time of intracytoplasmic sperm injection. Fertil Steril, 2013; 99: 400–7
36. Shirato K, Yotsumoto F, Itoh H et al: Separation efficiency of a microfluidic sperm sorter to minimize sperm DNA damage. Fertil Steril, 2016; 105: 315–21
37. Wang W, Liang GT, Peng YY et al: Effects of a microfluidic sperm sorter on sperm routine parameters and DNA integrity. Zhonghua Nan Ke Xue Zazhi, 2017: 301–4
38. Nosratí R, Vollmer M, Eamer L et al: Rapid selection of sperm with high DNA integrity. Lab Chip, 2014; 14: 1142–50
39. Tunc O, Tremellen K: Oxidative DNA damage impairs global sperm DNA methylation in infertile men. J Assist Reprod Genet, 2009; 26: 537–44
40. Hammadeh ME, Hamad MF, Montenarh M, Fischer-Hammadeh C: Protamine contents and P1/P2 ratio in human spermatozoa from smokers and non-smokers. Hum Reprod, 2010; 25: 2708–20

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Competing interests

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41. Yu B, Qi Y, Li D et al: Cigarette smoking is associated with abnormal histone-to-prolamine transition in human sperm. Fertil Steril, 2014; 101: 51–57
42. Houshdaran S, Cortesius VK, Siegmund K et al: Widespread epigenetic abnormalities suggest a broad DNA methylation erasure defect in abnormal human sperm. PLoS One, 2007; 2: e1289
43. Rappa KL, Rodriguez HF, Hakkarainen GC et al: Sperm processing for advanced reproductive technologies, Where are we today? Biotechnol Adv, 2016; 34: 578–87
44. Doane TL, Burda C: The unique role of nanoparticles in nanomedicine: Imaging, drug delivery and therapy. Chem Soc Rev, 2012; 41: 2885–911
45. Vasquez ES, Feigang JM, Willard ST et al: Bioluminescent minisperm as an imaging agent for mammalian spermatozoa. J Nanobiotechnology, 2016; 4: 20
46. Odhiambo JF, Sutovsky M, Delarenty JM et al: Adaptation of ubiquitin-PNA based sperm quality assay for semen evaluation by a conventional flow cytometer and a dedicated platform for flow cytometric semen analysis. Theriogenology, 2011; 76: 1168–76
47. Sutovsky P, Aarabi M, Miranda-Vizuete A, Oko R: Negative biomarker based male fertility evaluation: Sperm phenotypes associated with molecular-level anomalies. Asian J Androl, 2015; 17: 554–60
48. Lafuente D, Garcia T, Blanco J et al: Effects of oral exposure to silver nanoparticles on the sperm of rats. Reprod Toxicol, 2016; 60: 133–39
49. Moretti E, Terzuli G, Renieri T et al: In vitro effect of gold and silver nanoparticles on human spermatozoa. Andrologia, 2013; 45: 392–96
50. Degheidy T, Abdelfattah H, Seif A et al: Magnetic activated cell sorting: An effective method for reduction of sperm DNA fragmentation in varicocele men prior to assisted reproductive techniques. Andrologia, 2015; 47: 892–96
51. Romany L, Garrido N, Cobo A et al: Obstetric and perinatal outcome of babies born from sperm selected by MACS from a randomized controlled trial. J Assist Reprod Genet, 2016; 34: 201–7
52. Odhiambo JF, Delarenty J, Geary TW et al: Increased conception rates in beef cattle inseminated with nanopurified bull semen. Biol Reprod, 2014; 91: 97
53. Cross NL, Watson SK: Assessing acrosomal status of bovine sperm using fluoresceinated lectins. Theriogenology, 1994; 42: 89–98
54. Mulugeta Achame E, Wassenaar E, Hoogerbrugge JW et al: The ubiquitin-conjugating enzyme Hrb6 is required for maintenance of X chromosome silencing in mouse spermatocytes and spermatids. BMC Genomics, 2010; 11: 367
55. Conaway RC, Brower CS, Conaway JW: Emerging roles of ubiquitin in transcription regulation. Science, 2002; 296: 1254–58
56. Da Silva N, Barton CR: Macrophages and dendritic cells in the post-testicular environment. Cell Tissue Res, 2016; 363: 97–104
57. Richburg JH, Myers JL, Bratton SB: The role of E3 ligases in the ubiquitin-prolamine transition in human sperm. Fertil Steril, 2014; 101: 51–57
58. Sutovsky P, Moreno R, Ramalho-Santos J et al: A putative, ubiquitin-dependent mechanism for the recognition and elimination of defective spermatozoa in the mammalian epididymis. J Cell Sci, 2011; 114: 1665–75
59. Sutovsky P, Terada Y, Schatten G: Ubiquitin-based sperm assay for the diagnosis of male factor infertility. Hum Reprod, 2001; 16: 250–58
60. Ozarton C, Chouteau J, Sutovsky P: Clinical adaptation of the sperm ubiquitin tag immunosassay (SUTI): relationship of sperm ubiquitylation with sperm quality in gradient-purified semen samples from 93 men from a general infertility clinic population. Hum Reprod, 2005; 20: 2271–78
61. Eskandari-Shahraji M, Tavallaee M et al: Proper ubiquitination effect on the fertilization outcome post-ICSI. Andrologia, 2013; 45: 204–10
62. Kita Muram A, Miyachi N, Hamada H et al: Epigenetic alterations in sperm associated with male infertility. Congenit Anom (Kyoto), 2015; 55: 133–44
63. Manochants C, Chiamanchya C, Sobhon P: Relationship between chromatin condensation, DNA integrity and quality of ejaculated spermatozoa from infertile men. Andrologia, 2012; 44: 187–99
64. Setti AS, Braga DP, Vingris L et al: Sperm morphological abnormalities visualised at high magnification predict embryonic development, from fertilization to the blastocyst stage, in couples undergoing ICSI. J Assist Reprod Genet, 2014; 31: 1533–39
65. Zhang Y, Hong H, Cai W: Imaging with Raman spectroscopy. Curr Pharm Biotechnol, 2010; 11: 65–70
66. Huang Z, Chen G, Chen X et al: Rapid and label-free identification of normal spermatozoa based on image analysis and micro-Raman spectroscopy. J Biophotonics, 2014; 7: 671–75
67. Huser T, Orme CA, Hollars CW et al: Raman spectroscopy of DNA packaging in individual human sperm cells distinguishes normal from abnormal cells. J Biophotonics, 2009; 2: 322–32
68. Swain RJ, Stevens MM: Raman microspectroscopy for non-invasive biochemical analysis of single cells. Biochem Soc Trans, 2007; 35: 544–49
69. Mallidis C, Sanchez V, Wistuba J et al: Raman microspectroscopy: Shining a new light on reproductive medicine. Hum Reprod Update, 2014; 20: 403–14
70. Liu Y, Zhu Y, Li Z: Application of Raman spectroscopy in Andrology: Non-invasive analysis of tissue and single cell. Trans Androl Urol, 2014; 3: 125–33
71. De Luca AC, Managò S, Ferrara MA et al: Non-invasive sex assessment in bovine semen by Raman spectroscopy. Laser Physics Letters, 2014; 11
72. Li XY, Wang M, Chen JH et al: Flow cytometric and near-infrared Raman spectroscopic investigation of quality in stained, sorted, and frozen-thawed buffalo sperm. Anim Reprod Sci, 2016; 170: 90–99
73. Meister K, Schmidt DA, Bründermann E, Havenith M: Confocal Raman microscopy as an analytical tool to assess the mitochondrial status in human spermatozoa. Analyst, 2010; 135: 1370–74
74. Sánchez V, Redmann K, Wistuba J et al: Oxidative DNA damage in human sperm can be detected by Raman microspectroscopy. Fertil Steril, 2012; 98: 1124–29
75. Ferrara MA, Di Caprio G, Managò S et al: Label-free imaging and biochemical characterization of bovine sperm cells. Biosensors (Basel), 2015; 5: 141–57
76. Anel-López L, García-Álvarez O, Parrilla I et al: Effect of sex-sorting and cryopreservation on the post-thaw sperm quality of Iberian red deer spermatozoa. Theriogenology, 2017; 89: 206–13
77. Liu F, Zhu Y, Liu Y et al: Real-time Raman microspectroscopy scanning of the single live sperm bound to human zona pellucida. Fertil Steril, 2013; 99: 684–89
78. Poplineau M, Trussardi-Régnier A, Happillon T et al: Raman microspectroscopy detects epigenetic modifications in living Jurkat leukemic cells. Epigenomics, 2011; 3: 785–94
79. Schulze HG, Konorov SO, Caron NJ et al: Assessing differentiation status of human embryonic stem cells noninvasively using Raman microspectroscopy. Anal Chem, 2010; 82: 5020–27
80. Sundararajan N, Mao D, Chan S et al: Ultrarapid detection and characterization of posttranslational modifications using surface-enhanced Raman spectroscopy. Anal Chem, 2006; 78: 3543–50
81. van der Heijden GW, Ramos L, Baart EB et al: Sperm-derived histones contribute to zygotic chromatin in humans. BMC Dev Biol, 2008; 8: 34
82. Yuan S, Schuster A, Tang C et al: Sperm-borne miRNAs and endo-siRNAs are important for fertilization and preimplantation embryonic development. Development, 2016; 143: 635–47
83. Berdasco M, Esteller M: Genetic syndromes caused by mutations in epigenetic genes. Hum Genet, 2013; 132: 359–83
84. Hirasaawa R, Feil R: Genomic imprinting and human disease. Essays Biochem, 2010; 48: 187–200
85. Oviedo C, Abarca R, Lugones A et al: Spectroscopic analysis of the boundary-following sperm. Lab Chip, 2016; 16: 2418–22
86. Lopez-Garcia MD, Monson RL, Haubert K et al: Sperm motion in a microfluidic fertilization device. Biomed Microdevices, 2008; 10: 709–18
87. Zhong HZ, Lv FT, Deng XL et al: Evaluating HZAK in spermatozoa from male infertility patients. Fertil Steril, 2015; 104: 574–81
88. Aston KL, Uren PJ, Jenkins TG et al: Averant sperm DNA methylation predicts male fertility status and embryo quality. Fertil Steril, 2015; 104: 1388–97