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Understanding the Epigenetic Modifications in Sperm Genome

Eswari Beeram

Abstract

Sperm genome condensation is mainly important as the genome expression should be repressed until it is fertilised with oocyte. In most of the mammals, protamines play an important role in genome condensation. In humans, histone variants were present in germ cells when compared with somatic cells. How successful replacement of histones by protamines occurs in most of the mammals is an interesting question. Little information is known about transition proteins that replace histones with protamines. Apart from condensation, which mechanisms prevent expression of X and Y chromosomes in sperm is to be studied. If exposed to genotoxic agents like Metosartan that damage testes should also be considered, in order for assisted reproductive technologies like in vitro fertilisation to succeed.

Keywords: assisted reproductive technology (ART), intra cytoplasmic sperm injection (ICSI), invitro fertilisation (IVF), stem cell factor (SCF), protein dependent kinase (PDK), glial cell derived neurotropic factor (GDNF), post translational modification (PTM), chromatin assembly factor (CAF), testes specific serine/threonine kinase 6 (TSSK6), nucleolar organising region (NOR), p- element induced wlmphy testes in drosophila (PIWI), a disintegrin and metalloprotease (ADAM)

1. Introduction

Spermiogenesis involves several steps and finally differentiate to A spermatogonia from stem cells [1]. Self renewal of stem cells was necessary for production of sperm cells throughout the life. C-Kit and SCF play an important role in determining the germ cell fate. Retinoic acid receptor plays a crucial role in proliferation of germ cells. Expression of Proteins like PP2A is reduced in later stages of the spermiogenesis and differentiation of A- spermatozoa in to B spermatozoa occurs through various intermediate stages like A_{pr} , A_{al} , A_{1-4} and finally to B-spermatozoa. Commitment to B- spermatozoa was followed by meiosis-I and ii leading to formation of round spermatids. During the separation of chromosomes, cohesins hold the sister chromatids and disassemble after the homologous recombination leading to separation of chromosomes, defects in which leads to stage IV arrest. The round spermatids undergo several morphological and cytological changes like elongation of sperm head, acrosome formation and shedding of the cytoplasm. During the process, several signalling mechanisms like PDK-Akt pathway, GDNF-GFR1 pathway, mTOR pathway, and MAP kinase pathways operate proceeding to spermiogenesis.

2. Protamines are originated from histone H1

In humans and mammals histones was replaced by either protamines or histone variants in sperm cell. But recent discovery of protamine P1 show both N- terminal and C- terminal sequence similarity between each other, and where as histone variant precursor resembled to that of protamine P2. Histones are basic proteins rich in Arg and Lys residues where as protamines in *Styela* contain polyarginine tracts than compared with histones.

Sperm condensation is one of the important criteria to be considered during assisted reproductive technologies in order to prevent genetic defects in upcoming generation. Spermatid formation involves 12–14 steps, and finally elongation of nuclear material leads to elongated sperms with acrosome. In sperm, histones are replaced by protamines and in humans histone variants are present leading to sperm condensation. Disulfide bonds contribute additional stability to the condensation as it results in interlinking of protamines [2, 3]. Certain PTMs of histones was also one of the epigenetic modifications required for condensation. Decondensation of Sperm chromatin takes place after sperm penetration in to oocyte which leads to exposure of sperm DNA [4] to oocyte reducing agents like glutathione. As known, glutathione reduces disulfide bonds between the protamines and relaxing the sperm DNA. From the previous reports, agents like SDS, EDTA reduces the disulfide bonds in combined state. Glycosaminoglycans like heparin sulphate binds to receptors on sperm membrane and promotes decondensation instead with nonsulfated form heparin (**Figure 1**).

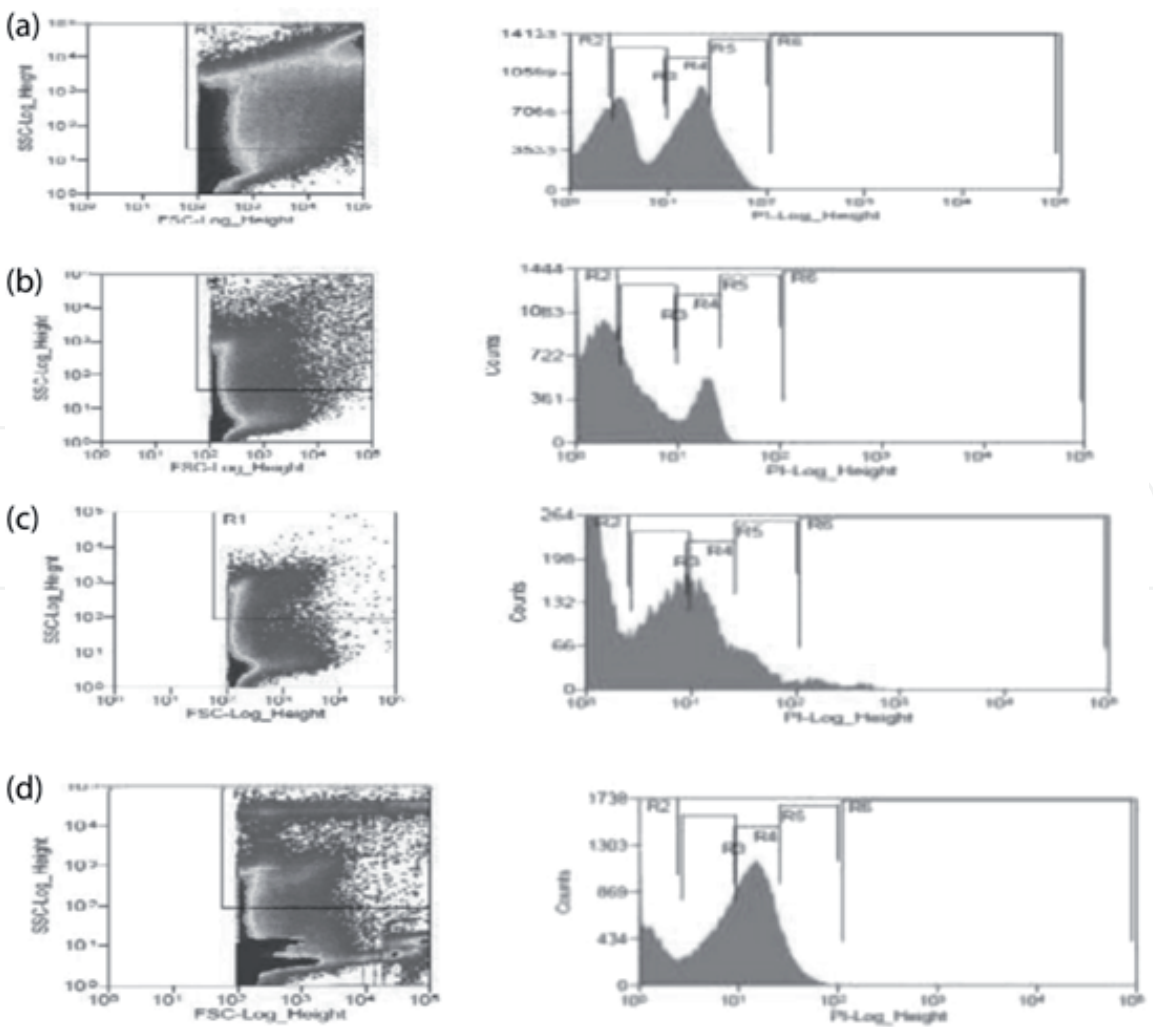


Figure 1. Flow cytometry of spermatozoa showing DNA fragmentation. Where (a) is control, (b) is testes treated with Metosartan (c) with RNaseA + aspirin and (d) with RNaseA+metosartan.

Acrosome and mitochondrial sheath formation occurs at 6 and 16 steps of the sperm biogenesis. Acrosome vesicles are secreted from the Golgi vesicles and form acrosomes cap around the nucleus. Defect with vesicle fusion or vesicle proteins leads to abnormalities in sperm head. Mitochondria is the major energy reserve of the sperm and the movement of mitochondria occurs through IMT pathways. Finally the sperms shed their cytoplasm by forming a cytoplasmic droplet around the neck region and remains attached to Sertoli cells until they become mature. Apical epistatic interaction involves holding of sperms at Sertoli junctions through the help of adherens proteins like NECTIN, Integrin- laminin and cadherin-catenin. Loss of Protein leads to premature release of sperms from Sertoli cells into the lumen of seminiferous tubules and abnormalities leads to vice versa of the same.

3. Testes-specific histones

H1T1 was one of the variant of linker histone found during pachytene of the cell division. H1T2 was the other linker variant found in apical pole of spermatids and responsible for male fertility. In case of humans HilS1 is necessary for condensation in elongated spermatids. Certain Core histone variants like TH2A and TH2B are found to be elevated during elongation of spermatids and whereas the normal core histones show low amounts of expression. Gene knock outs of TH2A and TH2B induced male infertility but PTMs on core histones compensate the loss of these variants in the spermatids [5]. TH2A was found to be present at transcriptional start site [6] and where as TH2B is required during leptotene of the cell division.

Other histone variant is with core histone H3 which include H3.3, H3T and CenP. H3.3 is present at the stage of spermatids [7] and where as H3T was specifically expressed in testes and both differ by 5 amino acid residues from core histone H3.1. H3.3 represents actively transcribed regions where as H3.1 represents transcriptionally repressive regions and may be involved in replacement of Histones by protamines. H3.1 may be required for maintaining the repression of X and Y chromosomes where as H3.1 is involved in activating the genes required for transition protein and protamine synthesis.

Recently, humans contain histone variants instead of canonical histones which are subjected to acetylation at H₄K5, H₄K8, H₄K12 and H₄K16. In *Drosophila* H3 is also acetylated especially at H₃ K9 by Plant domain containing protein PYGO2 and responsible for open confirmation of chromatin. Presence of histone chaperones as identified recently in *Drosophila* named CAF1 (Chromatin Assembly Factor) and in humans was normal Heat shock protein variant namely HSPA2 (HSP 70.2). These chaperones acts as chromatin remodellers and responsible for exchange of transition proteins with protamines. ATP dependent remodeller proteins that act as histone chaperones was not known up to now, but above mentioned are some of the remodellers that help in chromatin remodelling in testes.

4. Various histone modifications during spermiogenesis

Histone modifications like phosphorylation, acetylation and ubiquitination play an important role in spermiogenesis [8]. Phosphorylation is found to be common on the four core histone proteins, and acetylation was mostly seen in H4, whereas ubiquitination was with respect to H2A and B. Phosphorylation and acetylation leads to neutralisation of charges on histones whereas ubiquitylation causes Rnf 8 mediated recruitment of transition proteins and found to be normal in gene knock out animals with respect to meiosis and repression of expression in germ cells.

Methylation is one of the main PTM to be considered during spermiogenesis. Lysine methylation is the most common methylation in histones during spermiogenesis. To some extent arginine methylation by type iii arginine methylases was also one of the common methylation that occurs in protamines like PRM2. Methylations like H2BK117 and H2BK121 is required for TH2B replacement in germ cells. Methylation in H3K9 was required for maintaining chromatin in repressed state [9], and it helps in maintaining some residual nucleosome core histones in paternal genome seen in sperm. Phosphorylation of H2A by TSSK6 is required for histone acetylation followed by condensation of chromatin [10, 11]. May be DNA double strand breaks activate TSSK6 in late spermatids forming γ H2AX foci required for replacement of histones with protamines [12].

Poly ADP-ribosylation is one of the events involved in gene regulation and Cell proliferation. ADP-ribosylation was another important modification to be considered during meiotic programme and also during DNA condensation [13]. Both the steps requires ds break formation and as per recent reports poly ADP ribosylation was seen during stages of spermatocytes and spermatids but not in case of mature spermatids. Ds breaks activates enzyme Poly ADP ribosyl polymerase which was taken as a score of DNA damage that proceeds with ds break formation [14, 15].

Methylation, Ubiquitination, acetylation were some of the common modifications seen in sperm chromatin during late spermatid stages. However sumoylation is attributed to centromere and telomeric heterochromatin regions. Presence of canonical histones was necessary for the identification of the paternal genome by embryo. In late spermatid stage most of the genome mRNA is kept in repressed state where as Protamine and transition proteins synthesis occurs in early spermatocytes.

Phosphorylation is one of the PTM that protamine undergoes before its exchange with transition proteins. After loading on DNA dephosphorylation and disulfide bond formation of protamine occurs. Protamine P2 is synthesised as precursor and processed later on in order to get a functional protein. Disulfide bond formation mainly occurs during the transit of spermatozoa from caput to cauda. Thiol content has positive relation with tyrosine phosphorylation and infertility. Some of the residual Histones must be required in the sperm cells as it is necessary for gene expression and genetic imprinting of certain genes in paternal genome. Phosphorylation of protamines was effectively seen at some places and necessary for effective binding with DNA. Zinc stabilises the sperm chromatin mainly due to binding to protamines. Protamines interacts with toxic metals so pesticides used, damages DNA integrity by binding to protamines and some of them may also cause alkylation of protamines.

5. Nuclear basic proteins

Transition proteins TNP1 and TNP2 are involved in transition of histones to protamines. TNP2 was responsible for the condensation of DNA through interacting with PRM2 by methylating events. TNP1 and 2 are the basic proteins rich in lysine and arginine residues where as protamines consists of lysine and cysteines residues and loss of protein interactions between TNP2 and PRM2 results in prevention of histone replacement with protamines proved by spatiotemporal orientation of these genes on chromosome [16]. TNP1 is involved in decondensation of chromatin [17] at nucleosome core and relaxing DNA with the help of topoisomerase which removes positive supercoiling. TNP1 is also involved in repair of ss breaks of DNA generated during the chromatin remodelling. Histones are present as nucleosome core up to 11–12 step and replaced by TPs at 12–13 steps and by protamines in 13–15 steps. TNP1 and TNP2 are loaded on to DNA at apposition ends [18, 19] and the mutants of TNPs does not alter the expression of protamines PRM1 [20] and 2 (**Figure 2**).

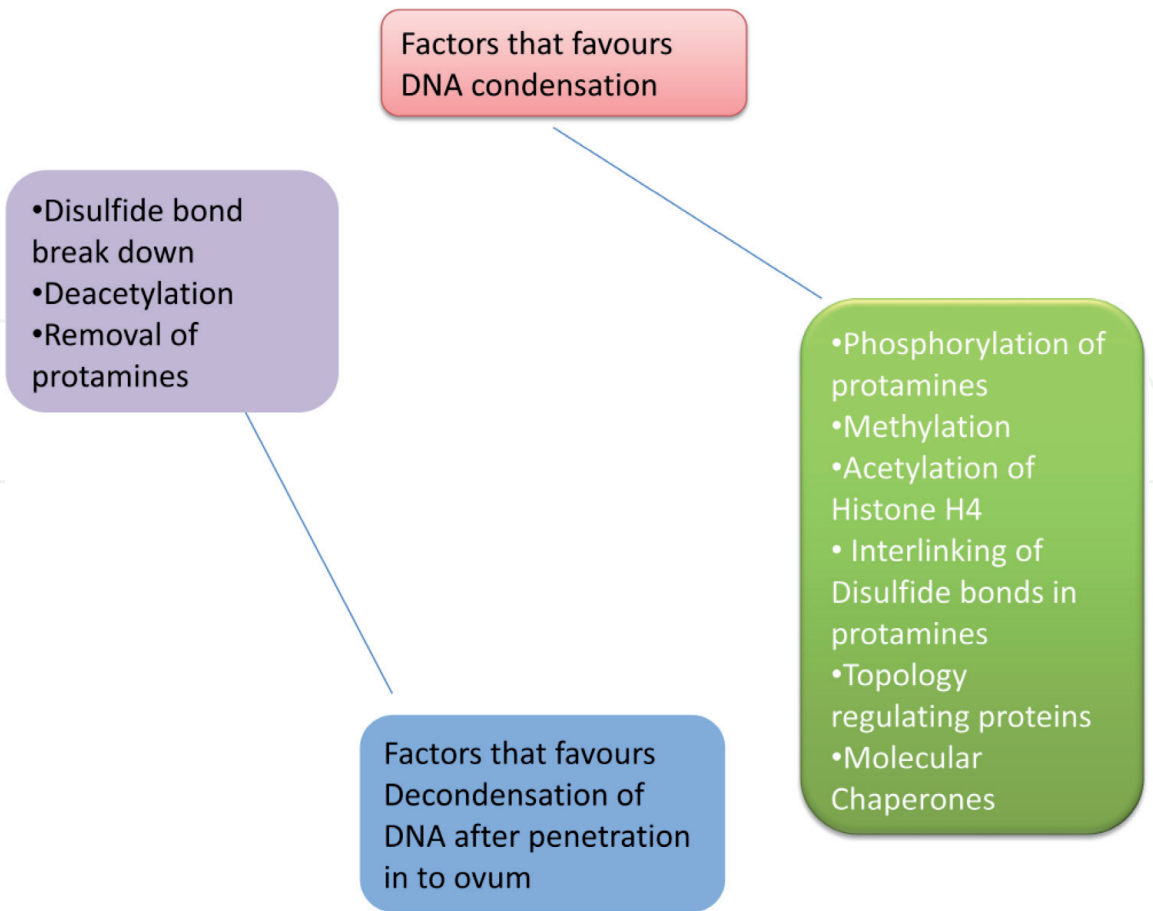


Figure 2.
Factors responsible for maintenance of genome integrity from parent to offspring.

Histone to protamine transition involves replacement of histones by protamines. From the previous reports, transition proteins play an important role in guiding this process. But recent report on male infertility has proved that ubiquitination of histones by RNF8 a ring domain containing proteins in the nucleus [21]. This protein mainly interacts with MIWI a PIWI protein and sequesters the protein RNF 8 in the cytoplasm. This interaction mainly prevents histone replacement by protamines even though the expression of protamines and transition proteins are normal. In this case not only the replacement of histones, but also the posttranslational modifications in protamines also play a major role in male fertility [22]. For example incorporation of Prm2 on chromatin requires phosphorylation of Prm2 to attain maturity of the protein so that it can interact with DNA. Phosphorylation of Prm2 is carried out by CAMK, loss of which leads to male infertility.

From the recent reports RNF8 is required for ubiquitination of H2A and H2B followed by H4 acetylation. Ubiquitinated H2 and H2B acts as platform for binding of MOF (Males absent of first) necessary for dosage compensation in drosophila fly and adds acetyl groups to H4 leading to free chromatin. The role of RNF8 is limited to spermatid stage and post meiotic, and plays an important role in male fertility. But how the ubiquitinated histones are protected from degradation by proteasome is a major question. But it is solved by spatial and temporal expression of proteasomal proteins especially in the late spermatid state.

Males defective in genes Tnp1 and Tnp2 does not effect the expression of proteins either protamines or Tnps. But abnormal retention of Tnp1 and 2 is the main reason for elevated levels in both Tnp-/- and as well as Tnp+/- mutants. The mutations would not affect the transition from histones to protamines. Histone to Protamine transition follows inter and intra disulfide bond formation in between

cysteines of protamines there by centering the amino and carboxy terminal ends with DNA binding domain. Disulfide bonds are necessary for further compaction of DNA and four of seven cysteines in protamines were involved in disulfide bond formation. Reduction of disulfide bonds leads to decondensation of chromatin proved by X-ray crystallography. Intra and inter linking of protamines through disulfide bond formation occurs at spermatid stage especially during maturation in epididymis [23], before to 14 days of their release.

Inhibin B activates mainly five pathways namely GPCR signalling, calcium pathway [24], MAP kinase pathway, PI3 pathway, Phospholipase A2 pathway. In which GPCR signalling, calcium Pathway and MAP kinase pathway causes phosphorylation of CAMP Responsive Element Modulator (CREM). This will leads to decreased expression of Prm2 causing male infertility. As earlier discussed histone—Protamine replacement requires certain modifications in histones like acetylation. In mouse CHD5 promotes H4 acetylation and ablation of this gene leads to retention of H3 and decreased condensation of nuclear DNA in head. This gene is expressed in both brain and testes but with higher in testes. Haploid insufficiency of gene does not have any effect on male fertility but the DNA condensation is affected further from step 9 of spermiogenesis [25] with out affecting brain tissue. Abnormality of sperm was mainly due to defects in condensation of chromatin [26] but not due to other defects like cytoplasmic retention, apical epistatic specialisation, acrosomes biogenesis, flagella movement and mitochondrial sheath formation.

6. ABP as the main molecule for chromatin condensation and decondensation

Androgen Binding Protein (ABP) acts as carrier for steroid hormone [27] Dihydrotestosterone the potent form of testosterone which acts as acceptor of hydrogen atoms. It oxidises disulfide bonds in the nucleus of sperm at the time of

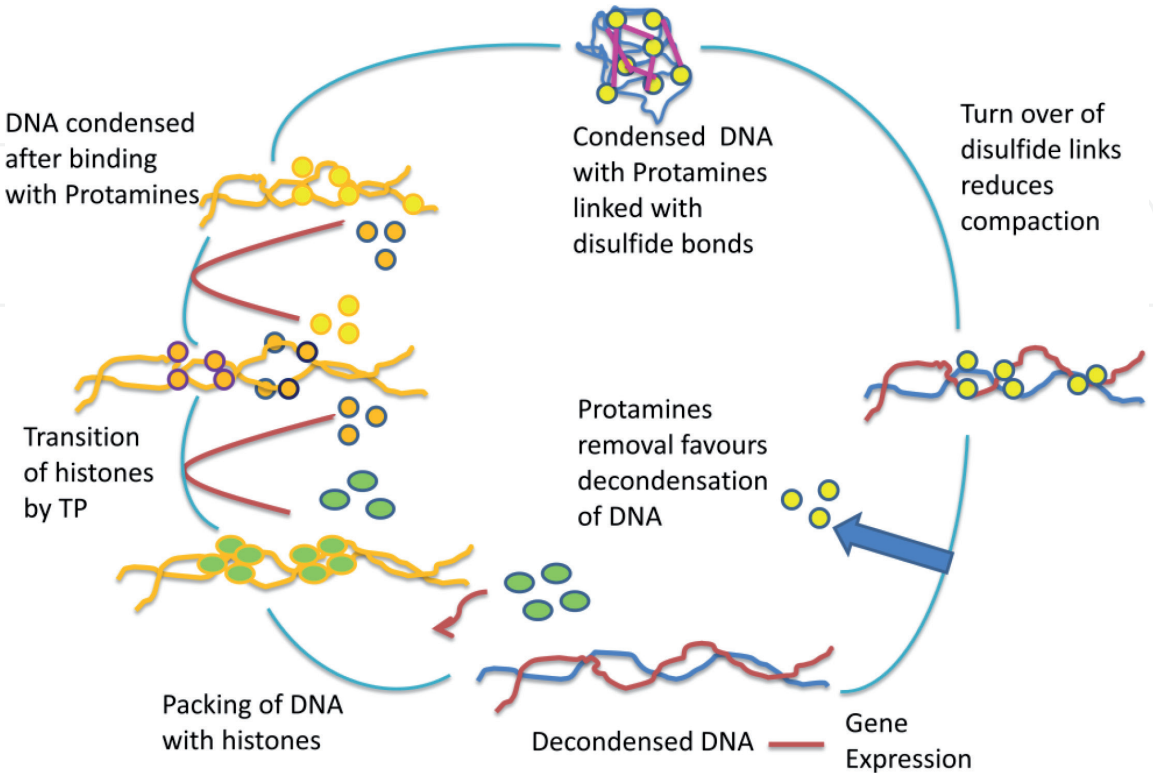


Figure 3. Overall schematic representation of condensation and decondensation of sperm DNA.

maturation in caput epididymis. 3- Hydroxy steroid dehydrogenase is the enzyme that has both oxidase and reductase activities but oxidase activity requires activation by female sex hormones like oestrogens or Progesterone. The enzyme requires NADPH as reducing equivalent and later glutathione after oocyte penetration to induce decondensation [2]. The process requires 3- diol instead of dihydrotestosterone. The steps of condensation are ABP > ABP—Dihydrotestosterone > Nuclear Localisation > Release of Dihydrotestosterone > Condensation > Penetration in to oocyte by sperm > Decondensation > Decondensation further By Glutathione > Reducing disulfide bonds in DNA (**Figure 3**).

7. Cell cycle analysis in spermatozoa using aniline blue

In S phase the dye uptake is more compared to G1 phase, where as similar in M phase as that of the S phase because immature sperms are stained by aniline blue and in case of aneuploid and apoptotic cells the dye uptake is low. By aniline blue we cannot distinguish the dividing cells from the cells undergoing segregation of the nuclear content followed by cytokinesis. The maximum absorption spectrum of aniline blue is 234.5 nm which can be used to detect the dye by flow cytometry using detector at this wavelength. It is low cost effective compared to propidium iodide but it has yet to be used and by this, we can study the aneuploidy, sperm maturity and apoptosis of sperm cells by using ejaculated semen sample. Flow cytometry analysis of sperm suspension collected from epididymis can be performed after staining with aniline blue as protamines exclude aniline blue, only immature sperms takes aniline blue so, this property of dye can be used to study sperm maturity by flow cytometry.

8. Understanding the topology of spermatocytes

Spermatocyte recombination mainly occurs by double strand break formation and involves interactions between chromosome domains by forming nuclear territories. The telocentric chromosomes along with NORs form bouquet like structure at the periphery of the nucleus where as metacentric chromosomes at centre of the nucleus. If $2n = 24$, 8 bivalents are formed and attached to form bouquet structure. There was little exchange seen between the nonhomologous chromosomes in *Mus* species so evolutionary importance is less in these species. Heterochromatin surrounding the centromere form the basis for absence of recombination as it encodes proteins that keep the centromere integrity.

Constitutive heterochromatin in nucleolus makes the rDNA arrangement with difference in different species. Together with proximal arrangement with respect to telomere and metacentric chromosomes with NORs in long arms serve as excellent models for study of both NORs and bouquet formation, seen in maintenance of topology. Bouquet formation is necessary for interaction of chromosomal domains in nuclear territories. Two telocentric chromosome forms a single metacentric chromosome which forms the basis of recombination.

EtBr is normally used as intercalating dye to study DNA and also to stain DNA. Increased concentrations of EtBr causes decondensation of DNA as the bonds between the intercalated bases rearrange and results in loop formation. After certain concentration of dye, the decondensed DNA will be condensed again, i.e., biphasic kinetics was seen with the epididymal sperms. In late elongated spermatids histones are already replaced by protamines as cysteines present in protamines provide extra stability and these sperms are proven to resistant to DNase I digestion, up to shorter time periods.

9. Molecular chaperones are necessary for male fertility

Molecular chaperones are the proteins, which help in folding of proteins and prevent their aggregation. So after spermiogenesis, post testicular maturation in requires proteins like chaperones in addition to several other proteins in epididymis. Those are the proteins like HSP60, HSP70 family including HSPA2, HSPA5, and other than HSP 70 family include chaperonin containing t- complex protein and HSP90. HSP 70 plays an important role in maintaining proteins in partially folded state and facilitates in transport of those proteins in the membranes. HSPA2 is responsible for spermiogenesis and also aids in maturation of sperms. It binds to the plasma membrane of sperm along with its co chaperones and forms heterocomplex with CDC2 and cyclin B during G1- S phase and G2- M phase transitions. This protein is also present in oviductal secretions along with HSPA5 and is necessary for capacitation of sperms.

As discussed previously the thiol content reflects the tyrosine phosphorylation. HSPA2 promotes condensation analogous to decondensation factor and terminates signalling by tyrosine phosphorylation. Chaperonin containing t- complex protein (CCT) binds to sperm plasma membrane and promotes ZP binding as it promotes binding to ZP receptors. CCT protein along with its co chaperones enters the sperm through endocytosis of receptor and ligand coated with caveolin protein in membrane rafts. This protein is mainly present in t-complex of sperm and prevents protein aggregation or misfolding. CCT is mainly composed of 8 subunits in which 3 subunits form the domains and substrate recognition was through electrostatic and hydrophobic interactions, and then transported to the central cavity of the protein.

HSP60 was the other molecular chaperone found in mitochondria and helps in transport of proteins in to mitochondria. HSPA5 also known as Bip aids in folding of proteins in endoplasmic reticulum and participates in ER stress signalling pathway. Like HSPA5 calcium binding protein Calnexin also express in ER and become arrested after late spermatid stage. HSPA8 the one of the member of HSP70 and was expressed ubiquitously and facilitates binding of sperm to ovum. So, injection of this protein with IVF may be useful as it is important for enhanced survival of sperm in vivo.

Calnexin and Calsporin were the variants of Calnexin and Calreticulin seen in testes and along with ADAM1A, ADAM2 and ADAM 3 were required for sperm capacitation by interacting with zona of oocyte and helps in sperm—oocyte interaction. Calreticulin is mainly necessary for calcium oscillations inside the sperm cells for hyper activation and capacitation. PDI (Protein Disulfide Isomerase) along with Calnexin is necessary for disulfide bond formation and remodelling in sperm.

10. Epigenetic modulations in sperm genome

Histone H3 undergoes demethylation at K9 due to loss of lysine demethylase in primordial germ cells after E7.5 days. In meiotic recombination histone mono, di tri methylations are responsible for DSBs and recombination. Methylation at pericentric regions [28] is responsible for preventing homologous recombination. Retrotransposons restrict methylating events and responsible for maintenance of genome imprinting. Reduced DNA methylation in retrotransposons leads to gene expression in LINE 1 transposons and proteins like PIWI and AUBERGINE undergoes methylation in repetitive elements of PGC and responsible for gene silencing mechanisms [29]. This pattern was found to be conserved in mice and in animals and mammals, in addition to mutational hotspots there are some recombination hotspots characterised by H3K4me3 and H3K9 acetylation.

Paternal genomes are hypomethylated compared to maternal genome and in *Drosophila* the protamine eviction was done by DHD (Dead Head) by reducing the disulfide bonds with the help of thioredoxin and NADPH. Protamines form oligomers by intrachain disulfide bonds. Molecular chaperone TAP/p32 acts on monomeric protamines and causes their eviction and found to be inactive on oligomers. DHD interacts physically with TAP/P32 to cause the eviction of protamines from DNA and TAP/P32 binds poorly to DNA. DHD normally found in sperm nucleus and was degraded after fertilisation. DHD domain was necessary for the reduction of disulfide bonds, DNA decondensation, and protamine eviction and also functions as chaperone. It shares some common properties with thioredoxins and also has unique properties necessary for the embryo formation. Mutation in DHD domain is non tolerant and results in haploid embryo formation with the absence of male pronucleus [30].

Proteomic profile of testes sperm was one of the important researches in progress nowadays. Normally in DNA condensing proteins the disulfide bonds are oxidised spontaneously and does not require any enzymatic source. There are certain proteins in sperm which require enzymes like thioredoxin/Glutathione reductase which acts on glutathione peroxidase and induces disulfide bond formation. Thioredoxin glutathione reductase catalyses isomerisation of disulfide bonds in these proteins and the protein was mainly localised at mid piece of mitochondrial sheath and was required for structural maintenance of mid piece and tail proteins. Those include proteins like mitochondrial capsule selenoprotein, two outer dense fibre (ODF) and glutathione-s-transferase M5. Differences in maternal and paternal chromosomes mainly occur at pericentric chromosomes, through which epigenetic message is conveyed to embryo.

11. Differentiation in meiotic events of sperm and oocyte in *C. elegans*

In oocyte, synaptonemal complex (SC) is formed along the lateral and central axis. In sperms condensation plate is formed after diplotene. Formation of karyosomes, [the aggregation of chromosomes] is characterised in both oocytes and sperms of *C. elegans* and other mammals and referred as karyosome stage in *C. elegans*. The meiotic process mainly differs in microtubule dynamics. In oocytes chromosome dependent spindle formation is necessary for chromosome segregation, where as in case of sperms centrioles are present. So, the spindle formation is mediated by centrioles.

There are some of the kinetochore differences observed in oocytes and sperms. In sperms, outer kinetochore proteins attract the inner proteins. CENP -c is present in spermatocytes and CENP- A is present in oocytes. Some of the kinases like AIR-2 is present in spermatocytes and PLK-1 is found in oocytes. SYP-1 is necessary protein of central element in SC and recruits AIR-2. Mutation in SYP-1 protein leads to random distribution of metaphase bivalents. Some of the kinases like AIR-2 phosphorylates Rec-8 protein which is required for cohesion of sister chromatids. Cytokinesis after meiosis -1 is incomplete in both sperms and oocyte without any necessity for re condensation of chromatin after diplotene.

12. Oxidative potential in testes and epididymis is required for spermiogenesis

GPRX 4 and PXR4 are the two enzymes which are mainly required for compaction of chromatin in sperm mainly by sulfoxidation of protamines attached to

DNA. PXRX4 is necessary for condensation in testes where as GPRX4 is specifically in epididymis. Hydrogen peroxide acts as signalling molecule and causes phosphorylation of tyrosine molecules in the receptor, necessary for proliferation of sperm cells [31]. Abnormal signalling by hydrogen peroxide leads to sperm cancer which can be avoided by reduction of H₂O₂ by enzymes like SOD and catalase. Totally GPRX4 and PXRX4 is necessary for nuclear stability, condensation and spermiogenesis.

13. Factors responsible for condensation and decondensation of chromatin

Zinc is well known for maintenance of membrane stability and found to be present in seminal plasma. It is decreased during penetration of sperm in to the female reproductive tract and decrease of zinc causes premature decondensation of chromatin as it destabilises the sperm plasma membrane. Albumin also binds to zinc and accelerates decondensation. In humans heparin is involved in decondensation of chromatin. Zinc also binds to free thiols and increases stability of chromatin, necessary for reducing disulfide bonds and accelerating the reaction (Figure 4).

Few of the recent techniques used for measurement of DNA condensation and decondensation include using of TPM motions and energy. Condensation of DNA leads to decrease in length as protamines are organised as toroids. Where as decondensed chromatin is more in length as protamines are replaced by histones. Differences in motions of the DNA attached to the coverslip is captured and ultimately the difference in energy. Positive relation is seen between the persons with premature decondensation in DNA and infertility and DNA damage based on the studies in human subjects. Sperm fluorescent in situ hybridisation is other technique used to assess the relation between sperm DNA [32] condensation and abnormalities of head.

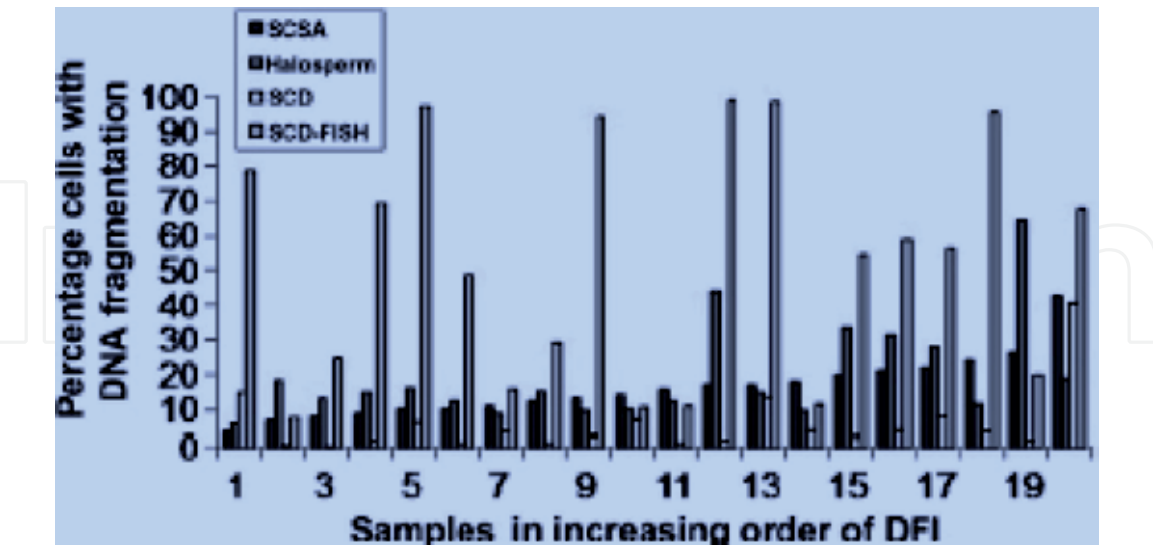


Figure 4. Percentage of cells with DNA fragmentation using different techniques of assessment.

14. Assisted reproductive technologies required for success of reproductive outcome

ICSI and IVF are the techniques used in assisted reproductive technology, in which ICSI resulted in more percentage of PCC (pre mature condensation of

chromatin) compared to IVF. It is mainly due to lack of activation of oocytes. and Pre mature condensation is due to the condensing factors released from the unactivated oocytes without affecting the sperms with intact plasma membrane. The main failure for ICSI is due to the injection of whole sperm in to oocytes. Fusion of oocytes and sperm membranes activates the oocytes lacking of which, was one of the main drawback seen in ICSI [33, 34]. Even damage to sperm membrane is another draw back seen with this technique.

Expression of decondensing factors like MPF is transient, as temporal expression is seen whereas in later stages sperm dependent factor SDF is mainly involved in activation of oocytes causing further decondensation of sperm chromatin. IVF does not include steps that cause damage to sperm DNA [35] and prevention of oocyte activation. The low rate of success is due to many reasons. One of the main reasons is DNA damage in spermatozoa. ART involves use of techniques which does not involve natural fertilisation. So, there may be chance of DNA damage. Sperm DNA lacks repair proteins that aids in protection against DNA damage. So, oocyte acts as source for repair proteins as it is active in gene expression. If the DNA damage overwhelms the oocyte capacity it leads to genetic aberrations in future generations. So, instead of choosing a single technique one has to get through the different techniques that help in selecting the single top spermatozoa.

When compared with high DNA damage the rate of pregnancy is more in case of IVF compared to ICSI. But in case of miss carriages rate it is more in ICSI compared to IVF. The reason is the post paternal factors play a pivotal role in repair of damage, and use of testicular sperm is helpful in ICSI if there is repeated miss carriages and low pregnancy rates. The assessment of sperm genome integrity is limited because different techniques used gives different outcomes. But SCSA is useful to assess testicular sperm for good outcome of results. Finally the outcome of ICSI cannot be predicted with respect to DNA condensation in both testicular sperm collected by biopsy and ejaculated spermatozoa in terms of pregnancy, cleavage and embryo formation.

15. Summary

Condensation of sperm genome was necessary for maintaining genome integrity by transition of histones by transition proteins which in turn by protamines. These proteins undergo some of PTM namely phosphorylation, Acetylation, poly ADP ribosylation and methylation. Further stability in interaction between DNA and protamine involves addition of disulfide bonds to form interlinking protamines. Dihydrotestosterone acts as hydrogen donor and acceptor in testes and necessary for maturation of sperms. Zinc acts as stabiliser of Genome through its interaction with protamines and toxicity due to metals responsible for male infertility [36] is due to interaction of these metal ions with protamines. So, safe ART is required to maintain genome integrity in offspring.

16. Metosartan acts as genotoxic agent of testes

Metosartan induces endometrial carcinoma in testes and causes changes in chromatin dynamics and causes prematurity in sperms. It causes competitive and non competitive inhibition of RNase present in male Wistar rats. It also decreases the sperm count, motility and induces abnormalities in sperm structure. It also causes apoptosis in both testes and sperm by increasing the permeability of mitochondria.

Abbreviations

ART	Assisted Reproductive Technology
ICSI	Intra Cytoplasmic Sperm Injection
IVF	In vitro Fertilisation
SCF	Stem Cell Factor
PDK	Protein Dependent Kinase
GDNF	Glial cell Derived Neurotropic Factor
PTM	Post Translational Modification
CAF	Chromatin Assembly Factor
TSSK6	Testes Specific Serine/Threonine Kinase 6
NOR	Nucleolar Organising Region
PIWI	P-element–induced wimpy testes in Drosophila
ADAM	A Disintegrin and Metalloprotease

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