Chromosome positioning and male infertility: it comes with the territory

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Abstract

The production of functional spermatozoa through spermatogenesis requires a spatially and temporally highly regulated gene expression pattern, which in case of alterations, leads to male infertility. Changes of gene expression by chromosome anomalies, gene variants, and epigenetic alterations have been described as the main genetic causes of male infertility. Recent molecular and cytogenetic approaches have revealed that higher order chromosome positioning is essential for basic genome functions, including gene expression. This review addresses this issue by exposing well-founded evidences which support that alterations on the chromosome topology in spermatogenic cells leads to defective sperm function and could be considered as an additional genetic cause of male infertility.

Keywords Chromosome territories · Gene regulation · Human spermatogenesis · Male infertility

Spermatogenesis and infertility

Spermatogenesis is a complex and highly regulated process directed toward the efficient production of large numbers of spermatozoa from a renewable spermatogonial stem cell population. This process takes place in the seminiferous tubules of the testis, where spermatogonia proliferate, spermatocytes undergo meiosis, and round spermatids differentiate into spermatozoa. The proper progression of spermatogenesis requires a spatially and temporally regulated gene-expression pattern [1–5], which in case of alterations would affect the fertility status of individuals. Expression pattern alterations could arise from different ways. One of the most prominent is genetic alterations such as numerical anomalies of sex chromosomes involved in Klinefelter syndrome [6], Y-chromosome deletions [7] and pathogenic variants of crucial spermatogenic genes [8]. In this regard, alterations of gene expression profiles during meiosis have been associated with male infertility in several knockout mouse models [9]. Another source of variations has been related to changes of the sperm epige-

nome. Its analysis has demonstrated that events occurring during spermatogenesis can result in altered epigenetic marks, which generates a footprint of important relevance for early embryonic events [10, 11]. In recent years, alterations of the nuclear chromosomal topology of germ cells and spermatozoa have also been related to male infertility [12–17]. In this context, studies of the spatial distribution of chromosomes in spermatogenic germ cells and its association to the genome regulation are acquiring great relevance.

In this review, we first provide a general overview of chromosome territories (CTs) focusing on the factors that influence the chromosome positioning and their biological functions. We then address the review of the results of CTs distribution in human spermatogenic cells, specially focusing on the relationship between chromosomal position, gene regulation, and male infertility.

Chromosome territories and influencing organization factors

A basic feature of the nuclear architecture in somatic cells is the spatial organization of chromatin in CTs [18], which are defined as discrete regions of the nuclei occupied by specific chromosomes. The existence of CTs was predicted at the end of the nineteenth century in interphase nuclei of animal cells [19]. In 1909, Boveri introduced the term of CT based on a study performed on blastomere nuclei of the horse roundworm

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Parascaris equorum eggs [20]. Seven decades later, Cremer and collaborators experimentally demonstrated the existence of this organization [21]. In Cremer's study, a very narrow laser light was used to induce DNA damage in the cell nucleus. To repair the damaged DNA, cells incorporated radioactively labeled nucleotides present in the culture media. When chromosomes took on a condensed appearance in the next mitotic division, repaired regions (with radioactively labeled nucleotides) were detected by radiography. Interestingly, only a few chromosomes per cell were damaged, which supported the CT model suggested by Rabl [19].

In the last two decades, the advances in microscopy, FISH-based methodologies, and the development of chromosome conformation capture-on-chip (4C) technology have enabled

an accurate analysis of interphase chromosome positioning, providing new evidences for the non-random distribution of chromosomes in somatic nuclei [22–26] (Fig. 1). These evidences are based both on relative positioning between different CTs and on its internal organization.

Regarding the relative position of CTs, it has been reported that CTs are commonly arranged according to gene density: gene-rich chromosomes (+) are located nearer the nuclear center while gene-poor ones are preferably located at the periphery [27–31] (Fig. 1a). The influence of radial arrangement according to gene density not only accounts for the whole chromosome itself, but also for discrete chromosome regions [29]. Several data indicate that the influence of gene density on the radial nuclear positions of chromosomes has been

Fig. 1 Influencing factors on the chromosome distribution in somatic cell nuclei. **a** Gene density: gene-rich chromosomes (+) are located nearer the nuclear center and gene-poor chromosomes (−) are located at the periphery. **b** Chromosome size: small chromosomes are located at the nuclear center and large chromosomes at the periphery. **c** Telomeres: telomeric chromosome regions interact with specific components of the lamina. **d** Nucleolus organizer regions (NORs): chromosomal regions located in the short arms of the human acrocentric chromosomes

interact constituting the nucleolus. **e** Inter-chromosomal contacts: the juxtaposition of regulatory gene expression elements residing on different chromosomes (Chr. A and Chr. B) promoting the intermingling of chromatin from different CTs. **f** Topologically associated domains (TADs): CTs show internal areas of coordinated transcriptional activity. The chromatin of chromosome A (in blue) and B (in yellow) show two TADs (A.1/A.2 and B.1/B.2, respectively)

evolutionary conserved suggesting their important role in the functionality of the nucleus [30, 32]. It has been suggested that this specific nuclear arrangement could enhance gene regulation (expression or repression) by grouping areas with a more potentially active transcription (gene-rich chromosomes) or more transcriptionally silent (gene-poor chromosomes) [33, 34]. Ioannou and Griffin (2011) proposed that the central location of gene-rich chromosomes may have functional implications in order to coordinate their accessibility to the transcriptional machinery [34]. Despite this, it cannot be ignored that some gene-poor chromosomes can congregate highly active genes compared with other chromosomes with a higher concentration of genes. For instance, the Y-human chromosome, which scarcely has a hundred genes, is highly active during spermatogenesis. Interestingly, most of these genes are congregated in the same chromosome region located at the q-arm near the centromere. So, in this particular case, the gene density in a chromosome region probably prevails over the whole chromosome gene density in its positioning.

Another parameter that has often been related with radial chromosome position is chromosome size: large chromosomes are located at the nuclear periphery while small ones located at the center [35, 36] (Fig. 1b). To explain the chromosome size-dependent distribution of human chromosomes, there exist two biophysical models that act in a complementary way: the mitotic preset model and the volume-exclusion model [35]. Regarding the mitotic model, since mitotic chromosomes are distributed as a wheel-shaped rosette with the centromeres positioned toward inside of the rosette [36], this allows large chromosomes to extend outward while small chromosomes become restricted in central positions. In fact, several authors have confirmed the central location of small chromosomes on a metaphase plate [37–39]. In addition, it has been also suggested that this size-dependence positioning would be maintained during G1 interphase [35]. Concerning the volume-exclusion model, as chromosomes have a defined volume and they must be included in the spherical space of the interphase nucleus limited by the nuclear envelope, each chromosome displays a limited ability to re-occupy the primary domain of another different sized chromosome. Therefore, this limitation in chromosome movement preserves the size-dependent distribution observed in mitotic nuclei.

Concerning the internal CTs organization, gene density, transcriptional activity, guanine-cytosine content (GC content), and early/late replication timing have been related to the chromatin spatial organization [18, 40]. The parameters that orchestrate the intra-CT organization have been associated with the optimization of strategies for replication, transcription, repairing, and splicing processes. The interdependence of all these positional determining parameters makes it difficult to dissect their specific weight within a given DNA segment. In this sense, it has been observed that the radial position for specific genes differs significantly

between different cell types [41]. On the other hand, Kupper and collaborators (2007) reported that the radial chromatin positioning is shaped by local gene density but not by gene expression [42]. Additionally, the variability of the results about the positioning shift of individual genes toward the nuclear interior upon their transcriptional activation adds more variables to the equation [43].

Additional features are recognized to also influence the chromosome positioning in the nucleus. For instance, telomeres play a crucial role (Fig. 1c), as indicated by telomere enrichment at the nuclear periphery. This peripheral enrichment has been described during post-mitotic nuclear assembly and a more internal telomere localization during the rest of the cell cycle [44]. It is well known that the inner membrane of the mammalian nucleus contains an organized network of proteins known as the lamina. This structure is formed by lamins and takes part in the regulation of telomere dynamics and maintenance [45]. It has been observed that the interaction between telomeres and the nuclear envelope is mediated by lamin B1 and the shelterin telomeric repeat-binding factor 1 (TRF1) [44]. The participation of some components of the nuclear envelope in chromosome regulation goes beyond its interaction with telomeres. For instance, interphase chromosome positioning also relies on large domains of chromatin specifically associated with lamina compounds named lamina-associated domains (LAD), which also contribute to stabilizing gene repression [46]. Moreover, extensive interactions between the nuclear pore complex and transcriptional repressed regions or enriched heterochromatin regions have been described in HeLa human cell line [47]. Other chromosome features that influence the CT organization are the nucleolar organizing regions (NORs) [48] which, in humans, are located on the sub-terminal region of the short arms of acrocentric chromosomes (Fig. 1d). In interphase nucleus, these regions organize the nucleolus, a specialized region involved in ribosomal RNA synthesis and ribosome assembly [49].

CTs and biological functions

Several authors suggest that nuclear territoriality favors specific interactions between distant genomic regions to activate or repress their transcriptional activities [50, 51]. In some cases, proper gene expression requires the juxtaposition of regulatory elements residing on different chromosomes which promote the chromatin intermingling from different CTs [52, 53] (Fig. 1e). Chromatin looping and localization of active genes at the interface of different CTs may facilitate loci interaction by forming inter-chromosomal contacts and, ultimately, resulting in changes in expression. Accordingly, a particular CT distribution pattern would facilitate physical interactions between different distal genomic regions that are coordinately regulated. Actually, it has been observed that

transcriptional activation of some gene causes its reposition in the territory, looping out and away from the center of its CT [54–57]. Otherwise, gene-repositioning movements decrease when transcription is inhibited [58], although this gene reposition does not occur in all cases [43].

Concerning the modulation of gene position within their corresponding CTs, the state of gene expression also plays an important role [59–61]. In higher eukaryotes, chromatin fibers are folded into different topologically associated domains (TADs) (Fig. 1f). These domains are more abundant within a chromosome than across several chromosomes and, interestingly, genes included in a given TAD show a coordinated transcriptional activity [62]. Several authors report TAD disruptions leading to pathological outcomes [reviewed by 62], which consolidate the role of TADs as functional element. Interestingly, it has been also described that the TADs' organization is similar between different cell types and is highly conserved across species [63], denoting their biological relevance [62]. The establishment of TADs allows the radial position of genes with regard to their expression and regulation. It has been reported that genes located at the periphery of their CT tend to be transcriptionally active, probably because, in this location, they are more accessible to the transcriptional machinery [64, 65].

Overall, the parameters that orchestrate chromosome positioning compartmentalize the nucleus in different functional areas. Specifically, regions with transcriptionally active chromatin (belonging to the same or different CTs) would be located in the center of the nucleus to be closer to transcription factors (TF), RNA polymerases, and other transcriptional regulatory elements [66]. In contrast, the transcriptionally inactive chromatin would be located at the periphery of the nucleus, close to intermediate filaments, resident proteins, and the inner nuclear membrane and lamina. Therefore, the non-random chromosomal position enables a time-space control of gene transcription and regulation which is indispensable to the functionality of the cell.

Chromosome territories in human spermatogenesis

In view of the functional implications of the chromatin organization in the nucleus, it is not surprising that the nuclear distribution of CTs differs according to the tissue. For instance, Parada and collaborators (2004) described specific chromosome distribution in mouse cells obtained from different tissues [67]. It has also been reported that this nuclear distribution of CTs shows tissue-origin specificity [68]. That is, chromosome relative position is equivalent between lung fibroblast cell lines, but strikingly different in skin- and colon-derived human fibroblasts [68].

Studies of the nuclear architecture in human male germ cells are sparse due to several limitations. One of the most relevant is related with the samples obtaining, since testicular samples from fertile subjects are not easily available and the whole process is ethically challenging. Furthermore, several cell types are present in the human testis (somatic and germ cells at different stages of development) requiring cell-sorting methodologies to obtain enrich cell fractions. Despite this, published data provide enough evidences to support a non-random chromosome distribution in spermatogenic cells (Fig. 2).

Data from mice model have showed that the nuclear architecture of prophase I spermatocytes is determined by the mechanisms of meiotic pairing and recombination [69] (Fig. 2a). The assembly of synaptonemal complexes keeps the homologous chromosomes in the same nuclear region and their distribution depends on the attachment of the chromosomes to the nuclear envelope, mainly by their telomeric regions. In this context, it is expected that small chromosomes will be retained at the nuclear periphery because these chromosomes are not long enough to invade the central region of the nucleus [69]. In mammalian pachytene cells, including humans, the attachment of chromosomes to the nuclear envelope also affects the position of the nucleolus [70] and the position of the XY pair [71].

FISH studies in human pachytene nuclei have also shown the preferential proximity between specific groups of bivalents, in particular, the bivalent formed by chromosomes 15 and the XY bivalent [72, 73]. Moreover, FISH studies performed in metaphase I spermatocytes, in which homologous chromosomes still are in pairs, also show preferential proximity between the bivalents 15 and 22 and the pair XY [74], suggesting that chromosome distribution in the metaphase plate is not random. Taking into consideration that, in these stages (pachytene and metaphase I), chromosomes are paired through their homologous regions, this preferable association between non-homologous chromosomes has been related to a similar sequence identity (e.g., between the heterochromatic regions of the Y-chromosome and acrocentric chromosomes, specifically the non-centromeric heterochromatin of chromosome 15) [75]. A possible consequence of this high-order spatial organization of the genome could be the fact that 70% of the human Y-autosome translocations are produced between the Yq heterochromatin and the short arm of acrocentric chromosomes [76] (specially chromosome 15; 52%) [77]. It is generally assumed that inter-chromosomal translocations result from DNA double-strand breaks repair mechanisms between two different chromosomes positioned next to each other in the nucleus. Additional results also confirm the non-random chromosome distribution in metaphase I and demonstrate that this territoriality is influenced by chromosome size, gene density, and acrocentric morphology [78] (Fig. 2b). Interestingly, the influencing parameters observed in this

Fig. 2 Influencing factors on the chromosome distribution in spermatogenic germ cells and spermatozoa. Ad: Type A (dark) spermatogonia; Ap: type A (pale) spermatogonia; B: type B spermatogonia;

L: leptotene spermatocytes; Z: zygotene spermatocytes; P: pachytene spermatocytes; Dp: diplotene spermatocytes; Dk: diakinesis spermatocytes

study [78] are in agreement with the position-determining parameters described in interphase somatic nuclei [27–31, 35, 36, 49].

Concerning the CTs in human sperm nucleus, a notable number of publications provides evidence that chromosomes are not randomly distributed [12, 15, 79–87] (Table 1). Interestingly, the evolutionary conserved, non-random chromosome positioning in mammalian sperm nuclei [88] suggests a functional relevance [89]. In 1995, Zalensky and collaborators [90] hypothesized the hairpin-loop model in which centromere domains were clustered into a single chromocenter positioned in the inner region of sperm nucleus while telomeres were exposed to the nuclear periphery joined into dimers. It appears that the chromocenter plays a fundamental role in sperm nuclear organization and it is involved in proper intranuclear chromosome positioning, with preferred CT localization [91, 92]. Moreover, the association between the p- and q- telomeres of the same chromosome implies that they must adopt loop structure [81, 82], promoting a local and bound position of each chromosome inside the nucleus. From the postulation of this model, several authors have presented supportive data [15, 79–82, 84, 91] (Fig. 2c). A recent study has redefined the former model showing a more segmented organization of centromeres and telomeres [87, 93]. According to this study, multiple chromocenters would occupy inner and peripheral regions of the nucleus, while telomere dimers would predominantly localize in intermediate, peripheral, and mid regions.

Sperm-FISH studies have also shown that chromosomes present a radial (center versus periphery) and longitudinal

(basal versus apical) distribution of CTs along the head-tail axis, describing a non-random CT distribution in the nucleus of male gametes [12, 15, 79–87] (Table 1). However, there is great variability between studies, either due to the number of spermatozoa analyzed, the selection of chromosomes studied, the criteria used to establish the radial and longitudinal nucleus segmentation, the type of DNA FISH probes used (centromeric, telomeric, locus-specific, arm-specific, or painting), the use of two- or three- dimensional analysis methodology, or the statistical analysis performed. Therefore, there is still no consensus in the establishment of a CTs model for the human sperm nucleus. From the different variables analyzed in these studies (Table 1), gene density appears to be the only clear determining factor in sperm nuclear distribution of CTs regarding the radial parameter [83, 85, 86]. That is, gene-rich chromosomes are situated in the interior of the sperm nucleus, while gene-poor chromosomes are associated to the periphery (Fig. 2c). Interestingly, this observation is in agreement with the chromosome features that determine bivalent positioning in human metaphase I spermatocytes [78] and somatic interphase nuclei [27–31].

Although the functionality associated to the chromosome organization of the sperm nucleus is largely unknown, different possibilities have been proposed [87]. This distribution could be required to adopt the particular sperm nuclear morphology, which is essential for the sperm movement within the female reproductive tract. Also, the fact that gene-rich chromosomes are located in the center of the nucleus could be related to protective mechanisms of the paternal genome from DNA damage. Finally, some authors have associated the

Table 1 FISH-based studies on chromosome distribution in the human sperm nuclei

Reference	Chromosomes analyzed	DNA FISH probes	Analysis	Non-random longitudinal position	Non-random radial position	CTs influencing factors
Hazzouri et al. 2000 [79]	13, X	Painting	3D	Yes	Not evaluated	Not evaluated
Zalenskaya and Zalensky 2004 [80]	2, 6, 7, 16, 17, X, Y, 1,6, X	Centromere-specific painting	2D	Yes	Yes	Not evaluated
Mudrak et al. 2005 [82]	1, 2, 5	Arm-specific	2D	Yes	Not evaluated	Not evaluated
Manvelyan et al. 2008 [83]	All	Multicolor banding	3D	Yes	Yes	Gene density chromosome size
Finch et al. 2008 [12]	18, X, Y	Centromeric	3D extrapolations of 2D data	Not evaluated	Yes	Not evaluated
Ioannou et al. 2011 [84]	1–4, 6–12, 15–18, 20, X, Y	Pericentromeric loci	3D extrapolations of 2D data	Not evaluated	Yes	Not evaluated
Millan et al. 2012 [85]	18, 19, 21, 22, X, Y	Painting	2D	Yes	Yes	Gene density
Mudrak et al. 2012 [86]	1, 3, 6, 17, 18, 19, X, Y, 1, 3, 17, 18, X, Y	Painting Pan-centromeric and pericentromeric	2D	Yes	Yes	Gene density
Alladin et al. 2013 [15]	17	Pan-centromeric	3D	Yes	Yes	Not evaluated
Ioannou et al. 2017 [87]	1,5,19	Pan-telomeric and pan-centromeric	2D and 3D	Not evaluated	Yes	Not evaluated

reproducible CTs organization of the sperm nucleus with the ordered reactivation of the paternal genome in the zygote [82].

Alterations of CTs organization in spermatogenesis and infertility

In view of all the data presented, there is no doubt that the nuclear topology in spermatogenic cells and spermatozoa shows a non-random chromosome organizational pattern. In this context, it is not daring to envisage that this specific arrangement is essential for regulating accurate gene expression to allow the proper progression of spermatogenesis and early embryo development.

Regarding the influence of CTs on spermatogenic progression, there is indirect evidence associating an altered chromosome position during spermatogenesis with male infertility [91]. For instance, Finch and collaborators (2008) reported that centromeres of chromosomes X, Y, and 18 occupied a central nuclear location in human spermatozoa from control donors, while sex chromosomes appeared randomly distributed in sperm from infertile men [12]. Altered distribution of chromosomes 15, 18, X, and Y has been observed in sperm from infertile patients with increased aneuploidy frequencies, suggesting a link between sperm chromosome territoriality and chromosome segregation [13]. Interestingly, the territorial organization of bivalents in metaphase I is partially modified by the presence of sex-chromosome univalents in infertile

males [17] (a pairing abnormality closely related to the risk of sex-chromosome sperm aneuploidy). Moreover, motile and immotile sperm populations from normozoospermic patients showed dissimilarity in the nuclear position of chromosome 17 and in the formation of the chromocenter, probably denoting defects in the normal chromosomal territoriality [15]. Other authors described modest differences in nuclear organization for centromeric loci when they compared fertile sperm donors and patients with severely compromised semen parameters (oligozoospermia) and high sperm aneuploidy rates [84]. With regard to individuals with altered karyotype, in which male infertility is a common issue [94], an altered nuclear chromosome positioning has been described in reciprocal translocation carriers [14]. Moreover, data from Robertsonian translocation carriers showed that the territorial positioning of bivalents in metaphase I spermatocytes changed in the presence of this reorganization [16].

Focusing on the relationship between sperm CTs and embryo development, several authors have suggested that an appropriate organization of sperm chromosomes is crucial for early embryo development, including the formation of the male pronucleus and the position of chromosomes in the first embryonic division [79, 82, 91, 95]. The association between CTs and embryo development goes from basic structural features, such as DNA packaging by protamines (protamination), to the organization of the chromosomes in specific territories. Altered protamination has been associated with altered sperm chromatin packaging and DNA

damage [93], and hence, the production of an altered sperm chromatin environment could affect embryo viability. Although some authors have reported a negligible effect on fertility [96], there are several studies which report an association between poor protamination and reduced fertilization rate [97–100] as well as poor embryo quality [97–101]. This suggests that basic structural features of the sperm chromosomes are crucial to embryo development. Concerning the effect of high order sperm chromosome organization on embryo development, it is well known that, when spermatozoa fertilize the oocyte, the sperm nuclear envelope breaks down and chromosomes interact with ooplasm factors. From this moment, protamines are gradually replaced by oocyte histones and chromatin becomes less condensed [85, 91]. Since this exposure occurs in a stepwise manner rather than simultaneously, the correct organization of the sperm chromatin determines the timing of gradual remodeling of specific regions responsible for gene activation patterns in the early embryo. In other words, chromosomal localization could determine the time at which sperm chromatin domains are decondensed and remodeled, allowing a certain degree of epigenetic control and influencing the subsequent paternal expression of genes in the embryo [88, 91, 102]. Supporting this proposal, it has been suggested that, after intracytoplasmic sperm injection (ICSI) in which sperm chromatin en bloc suddenly invades the oocyte nucleus, there is a delayed decondensation of the sub-acrosomal nuclear region [103]. In this scenario, it has been suggested that the timing of activation of the sex chromosomes, which are apically located in the sperm nucleus, could be affected [103]. This could explain, at least in part, the slightly increased rate of sex chromosomal anomalies reported in children born after ICSI [104].

Final remarks

- CTs distribution in spermatogenic cells and spermatozoa has been poorly explored. Nevertheless, preliminary data suggest that chromosomes are arranged in a specific manner along the different stages of spermatogenesis.
- The non-random distribution of chromosomes along spermatogenesis appears to be essential for germ-cell differentiation and early embryo development.
- Variations in chromosome distribution have been observed in spermatogenic cells and spermatozoa from infertile patients suggesting a relationship between chromosome positioning, genome regulation, and male infertility.
- Recent advances in FISH-based imaging, capture technologies of chromosome conformation, and cell-sorting methodologies, will allow a fine-scaling analysis of the chromosome territories in spermatogenic cells and

spermatozoa. Their application in well-designed studies will promote:

- Describing a comprehensive model of chromosome territories along spermatogenic cells.
- Defining the relationship between chromosome territories and genome regulation along spermatogenesis.
- Determining whether chromosome positional variations in infertile patients are the cause or the consequence of a compromised spermatogenesis.
- Uncovering the consequences that an abnormal chromosome distribution has for the progression of spermatogenesis and early embryo development.

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References

1. Kimmins S, Sassone-Corsi P. Chromatin remodelling and epigenetic features of germ cells. *Nature*. 2005;434:583–9. <https://doi.org/10.1038/nature03368>.
2. De Mateo S, Sassone-corsi P. Regulation of spermatogenesis by small non-coding RNAs: role of the germ granule. *Semin Cell Dev Biol*. 2014;29:84–92. <https://doi.org/10.1016/j.semcdb.2014.04.021>.
3. Shima JE, McLean DJ, McCarrey JR, Griswold MD. The murine testicular transcriptome: characterizing gene expression in the testis during the progression of spermatogenesis. *Biol Reprod*. 2004;71:319–30. <https://doi.org/10.1095/biolreprod.103.026880>.
4. Margolin G, Khil PP, Kim J, Bellani MA, Camerini-Otero RD. Integrated transcriptome analysis of mouse spermatogenesis. *BMC Genomics*. 2014;15:39. <https://doi.org/10.1186/1471-2164-15-39>.
5. da Cruz , Rodríguez-Casuriaga R, Santiñaque FF, Fariás J, Curti G, Capoano CA, Folle GA, Benavente R, Sotelo-Silveira JR, Geisinger A. Transcriptome analysis of highly purified mouse spermatogenic cell populations: gene expression signatures switch from meiotic-to postmeiotic-related processes at pachytene stage. *BMC Genomics* 2016;17:294.
6. Gravholt CH, Chang S, Wallentin M, Fedder J, Moore P, Skakkebaek A. Klinefelter syndrome - integrating genetics, neuropsychology and endocrinology. *Endocr Rev*. 2018;39:389–423. <https://doi.org/10.1210/er.2017-00212>.
7. Song SH, Chiba K, Ramasamy R, Lamb DJ. Recent advances in the genetics of testicular failure. *Asian J Androl*. 2016;18:350–5. <https://doi.org/10.4103/1008-682X.178857>.
8. Bracke A, Peeters K, Punjabi U, Hoogewijs D, Dewilde S. A search for molecular mechanisms underlying male idiopathic infertility. *Reprod BioMed Online*. 2018;36:327–39. <https://doi.org/10.1016/j.rbmo.2017.12.005>.
9. Smimova NA, Romanienko PJ, Khil PP, Camerini-Otero RD. Gene expression profiles of Spo11^{-/-} mouse testes with

- spermatocytes arrested in meiotic prophase I. *Reproduction*. 2006;132:67–77. <https://doi.org/10.1530/rep.1.00997>.
10. Hammoud SS, Nix DA, Hammoud AO, Gibson M, Cairns BR, Carrell DT. Genome-wide analysis identifies changes in histone retention and epigenetic modifications at developmental and imprinted gene loci in the sperm of infertile men. *Hum Reprod*. 2011;26:2558–69. <https://doi.org/10.1093/humrep/der192>.
 11. Campubí C, Salas-Huetos A, Aiese-Cigliano R, Godo A, Pons MC, Castellano G, et al. Spermatozoa from infertile patients exhibit differences of DNA methylation associated with spermatogenesis-related processes: an array-based analysis. *Reprod BioMed Online*. 2016;33:709–19. <https://doi.org/10.1016/j.rbmo.2016.09.001>.
 12. Finch KA, Fonseka KGL, Abogrein A, Ioannou D, Handyside AH, Thornhill AR, et al. Nuclear organization in human sperm: preliminary evidence for altered sex chromosome centromere position in infertile males. *Hum Reprod*. 2008;23:1263–70. <https://doi.org/10.1093/humrep/den112>.
 13. Olszewska M, Wiland E, Kurpisz M. Positioning of chromosome 15, 18, X and Y centromeres in sperm cells of fertile individuals and infertile patients with increased level of aneuploidy. *Chromosom Res*. 2008;16:875–90. <https://doi.org/10.1007/s10577-008-1246-2>.
 14. Wiland E, Zęgała M, Kurpisz M. Interindividual differences and alterations in the topology of chromosomes in human sperm nuclei of fertile donors and carriers of reciprocal translocations. *Chromosom Res*. 2008;16:291–305. <https://doi.org/10.1007/s10577-007-1194-2>.
 15. Alladin N, Moskvovtsev SI, Russell H, Kenigsberg S, Lulat AG, Librach CL. The three-dimensional image analysis of the chromocenter in motile and immotile human sperm. *Syst Biol Reprod Med*. 2013;59:146–52. <https://doi.org/10.3109/19396368.2013.772679>.
 16. Solé M, Blanco J, Valero O, Vergés L, Vidal F, Sarrate Z. Altered bivalent positioning in metaphase I human spermatocytes from Robertsonian translocation carriers. *J Assist Reprod Genet*. 2017;34:131–8. <https://doi.org/10.1007/s10815-016-0809-y>.
 17. Mayans C, Blanco J, Valero O, Vidal F, Sarrate Z. Unpaired sex chromosomes in metaphase I human spermatocytes locally modify autosomal bivalents positioning. *Asian J Andrology*. 2018;0:0. https://doi.org/10.4103/aja.aja_45_18.
 18. Cremer T, Cremer M. Chromosome territories. *Cold Spring Harb Perspect Biol*. 2010;2:a003889. <https://doi.org/10.1101/cshperspect.a003889>.
 19. Rabl C. *Über Zelltheilung*. *Morph Jb*. 1885;10:214–330.
 20. Boveri T. Die Blastomerenkerne von *Ascaris megalocephala* und die Theorie der Chromosomenindividualität. *Arch Zellforsch*. 1909;3:181–268.
 21. Cremer T, Cremer C, Baumann H, Luedtke EK, Sperling K, Teuber V, et al. Rabl's model of the interphase chromosome arrangement tested in Chinese hamster cells by premature chromosome condensation and laser-UV-microbeam experiments. *Hum Genet*. 1982;60:46–56. <https://doi.org/10.1007/BF00281263>.
 22. van de Werken HJ, de Vree PJ, Splinter E, Holwerda SJ, Klous P, de Wit E, de Laat W. 4C technology: protocols and data analysis. *Methods Enzymol*. 2012;513:89–112.
 23. Markaki Y, Smeets D, Fiedler S, Schmid VJ, Schermelleh L, Cremer T, et al. The potential of 3D-FISH and super-resolution structured illumination microscopy for studies of 3D nuclear architecture: 3D structured illumination microscopy of defined chromosomal structures visualized by 3D (immuno)-FISH opens new perspectives for studies of nuclear architecture. *BioEssays*. 2012;34:412–26.
 24. Paz N, Zabala A, Royo F, García-Orad Á, Zugaza JL, Parada LA. Combined fluorescent-chromogenic in situ hybridization for identification and laser microdissection of interphase chromosomes. *PLoS One*. 2013;8(4):e60238. <https://doi.org/10.1371/journal.pone.0060238>.
 25. Sawyer IA, Shevtsov SP, Dundr M. Spectral imaging to visualize higher-order genomic organization. *Nucleus*. 2016;7:325–38. <https://doi.org/10.1080/19491034.2016.1187344>.
 26. Cremer M, Schmid VJ, Kraus F, Markaki Y, Hellmann I, Maier A, et al. Initial high-resolution microscopic mapping of active and inactive regulatory sequences proves non-random 3D arrangements in chromatin domain clusters. *Epigenetics Chromatin*. 2017;10(1):39.
 27. Bridger JM, Boyle S, Kill IR, Bickmore WA. Re-modelling of nuclear architecture in quiescent and senescent human fibroblasts. *Curr Biol*. 2000;10:149–52. [https://doi.org/10.1016/S0960-9822\(00\)00312-2](https://doi.org/10.1016/S0960-9822(00)00312-2).
 28. Boyle S, Gilchrist S, Bridger JM, Mahy NL, Ellis JA, Bickmore WA. The spatial organization of human chromosomes within the nuclei of normal and emerin-mutant cells. *Hum Mol Genet*. 2001;10:211–9. <https://doi.org/10.1093/hmg/10.3.211>.
 29. Lukášová E, Kozubek S, Kozubek M, Falk M, Amrichová J. The 3D structure of human chromosomes in cell nuclei. *Chromosom Res*. 2002;10:535–48. <https://doi.org/10.1023/A:1020958517788>.
 30. Tanabe H, Habermann FA, Solovei I, Cremer M, Cremer T. Non-random radial arrangements of interphase chromosome territories: evolutionary considerations and functional implications. *Mutat Res*. 2002;504:37–45.
 31. Cremer M, Küpper K, Wagler B, Wizelman L, von Hase J, Weiland Y, et al. Inheritance of gene density-related higher order chromatin arrangements in normal and tumor cell nuclei. *J Cell Biol*. 2003;162:809–20. <https://doi.org/10.1083/jcb.200304096>.
 32. Neusser M, Schubel V, Koch A, Cremer T, Müller S. Evolutionarily conserved, cell type and species-specific higher order chromatin arrangements in interphase nuclei of primates. *Chromosoma*. 2007;116:307–20. <https://doi.org/10.1007/s00412-007-0099-3>.
 33. Meaburn KJ, Misteli T. Cell biology: chromosome territories. *Nature*. 2007;445:379–781. <https://doi.org/10.1038/445379a>.
 34. Ioannou D, Griffin DK. Male fertility, chromosome abnormalities, and nuclear organization. *Cytogenet Genome Res*. 2011;133:269–79. <https://doi.org/10.1159/000322060>.
 35. Sun HB, Shen J, Yokota H. Size-dependent positioning of human chromosomes in interphase nuclei. *Biophys J*. 2000;79:184–90. [https://doi.org/10.1016/S0006-3495\(00\)76282-5](https://doi.org/10.1016/S0006-3495(00)76282-5).
 36. Bolzer A, Kreth G, Solovei I, Koehler D, Saracoglu K, Fauth C, et al. Three-dimensional maps of all chromosomes in human male fibroblast nuclei and prometaphase rosettes. *PLoS Biol*. 2005;3:e157. <https://doi.org/10.1371/journal.pbio.0030157>.
 37. Wollenberg C, Kieffaber MP, Zang KD. Quantitative studies on the arrangement of human metaphase chromosomes. *Hum Genet*. 1982;60:239–48. <https://doi.org/10.1007/BF00303011>.
 38. Mosgoller W, Leitch AR, Brown JKM, Heslop-Harrison JS. Chromosome arrangements in human fibroblasts at mitosis. *Hum Genet*. 1991;88:27–33. <https://doi.org/10.1007/BF00204924>.
 39. Leitch AR, Brown JKM, Mosgoller W, Schwarzbacher T, Heslop-Harrison JS. The spatial localization of homologous chromosomes in human fibroblasts at mitosis. *Hum Genet*. 1994;93:275–80. <https://doi.org/10.1007/BF00212022>.
 40. Federico C, Cantarella CD, Di Mare P, Tosi S, Saccone S. The radial arrangement of the human chromosome 7 in the lymphocyte cell nucleus is associated with chromosomal band gene density. *Chromosoma*. 2008;117:399–410. <https://doi.org/10.1007/s00412-008-0160-x>.
 41. Heppner C, Mannes A, Merz J, Peters J, Dietzel S. Three-dimensional positioning of genes in mouse cell nuclei. *Chromosoma*. 2008;117:535–51. <https://doi.org/10.1007/s00412-008-0168-2>.

42. Küpper K, Kölbl A, Biener D, Ditttrich S, von Hase J, Thormeyer T, et al. Radial chromatin positioning is shaped by local gene density, not by gene expression. *Chromosoma*. 2007;116:285–306. <https://doi.org/10.1007/s00412-007-0098-4>.
43. Takizawa T, Meaburn KJ, Misteli T. The meaning of gene positioning. *Cell*. 2008;135:9–13. <https://doi.org/10.1016/j.cell.2008.09.026>.
44. Crabbe L, Cesare AJ, Kasuboski JM, Fitzpatrick JA, Karlseder J. Human telomeres are tethered to the nuclear envelope during postmitotic nuclear assembly. *Cell Rep*. 2012;2:1521–9. <https://doi.org/10.1016/j.celrep.2012.11.019>.
45. Burla R, La Torre M, Saggio I. Mammalian telomeres and their partnership with lamins. *Nucleus*. 2016;7:187–202. <https://doi.org/10.1080/19491034.2016.1179409>.
46. Kind J, Pagie L, de Vries SS, Nahidiazar L, Dey SS, Bienko M, et al. Genome-wide maps of nuclear lamina interactions in single human cells. *Cell*. 2015;163:134–47. <https://doi.org/10.1016/j.cell.2015.08.040>.
47. Brown CR, Kennedy CJ, Delmar VA, Forbes DJ, Silver PA. Global histone acetylation induces functional genomic reorganization at mammalian nuclear pore complexes. *Genes Dev*. 2008;22:627–39. <https://doi.org/10.1101/gad.1632708>.
48. Pliss A, Fritz AJ, Stojkovic B, Ding H, Mukherjee L, Bhattacharya S, et al. Non-random patterns in the distribution of NOR-bearing chromosome territories in human fibroblasts: a network model of interactions. *J Cell Physiol*. 2015;230:427–39. <https://doi.org/10.1002/jcp.24726>.
49. Németh A, Grummt I. Dynamic regulation of nucleolar architecture. *Curr Opin Cell Biol*. 2018;52:105–11. <https://doi.org/10.1016/j.ceb.2018.02.013>.
50. Cavalli G. Chromosome kissing. *Curr Opin Genet Dev*. 2007;17:443–50. <https://doi.org/10.1016/j.gde.2007.08.013>.
51. Hübner MR, Eckersley-Maslin MA, Spector DL. Chromatin organization and transcriptional regulation. *Curr Opin Genet Dev*. 2013;23:89–95. <https://doi.org/10.1016/j.gde.2012.11.006>.
52. Branco MR, Pombo A. Intermingling of chromosome territories in interphase suggests role in translocations and transcription-dependent associations. *PLoS Biol*. 2006;4:e138. <https://doi.org/10.1371/journal.pbio.0040138>.
53. Nguyen HQ, Bosco G. Gene positioning effects on expression in eukaryotes. *Annu Rev Genet*. 2015;49:627–46. <https://doi.org/10.1146/annurev-genet-112414-055008>.
54. Volpi EV, Chevret E, Jones T, Vatcheva R, Williamson J, Beck S, et al. Large-scale chromatin organization of the major histocompatibility complex and other regions of human chromosome 6 and its response to interferon in interphase nuclei. *J Cell Sci*. 2000;113:1565–76.
55. Williams RRE, Broad S, Sheer D, Ragoussis J. Subchromosomal positioning of the epidermal differentiation complex (EDC) in keratinocyte and lymphoblast interphase nuclei. *Exp Cell Res*. 2002;272:163–75. <https://doi.org/10.1006/excr.2001.5400>.
56. Ragoczy T, Telling A, Savado T, Groudine M, Kosak ST. A genetic analysis of chromosome territory looping: diverse roles for distal regulatory elements. *Chromosom Res*. 2003;11:513–25. <https://doi.org/10.1023/A:1024939130361>.
57. Galiová G, Bártová E, Kozubek S. Nuclear topography of β -like globin gene cluster in IL-3-stimulated human leukemic K-562 cells. *Blood Cells Mol Dis*. 2004;33:4–14. <https://doi.org/10.1016/j.bcmd.2004.03.006>.
58. Mahy NL, Perry PE, Gilchrist S, Baldock RA, Bickmore WA. Spatial organization of active and inactive genes and noncoding DNA within chromosome territories. *J Cell Biol*. 2002;157:579–89. <https://doi.org/10.1083/jcb.200111071>.
59. Misteli T. Beyond the sequence: cellular organization of genome function. *Cell*. 2007;128:787–800. <https://doi.org/10.1016/j.cell.2007.01.028>.
60. Geyer PK, Vitalini MW, Wallrath LL. Nuclear organization: taking a position on gene expression. *Curr Opin Cell Biol*. 2011;23:354–9. <https://doi.org/10.1016/j.ceb.2011.03.002>.
61. Solinhac R, Mompert F, Martin P, Robelin D, Pinton P, Iannuccelli E, et al. Transcriptomic and nuclear architecture of immune cells after LPS activation. *Chromosoma*. 2011;120:501–20. <https://doi.org/10.1007/s00412-011-0328-7>.
62. Roy SS, Mukherjee AK, Chowdhury S. Insights about genome function from spatial organization of the genome. *Hum Genomics*. 2018;12:8.
63. Dixon JR, Selvaraj S, Yue F, Kim A, Li Y, Shen Y, et al. Topological domains in mammalian genomes identified by analysis of chromatin interactions. *Nature*. 2012;485:376–80. <https://doi.org/10.1038/nature11082>.
64. Verschure PJ, van Der Kraan I, Manders EM, van Driel R. Spatial relationship between transcription sites and chromosome territories. *J Cell Biol*. 1999;147:13–24. <https://doi.org/10.1083/jcb.147.1.13>.
65. Bornfleth H, Edelmann P, Zink D, Cremer T, Cremer C. Quantitative motion analysis of subchromosomal foci in living cells using four-dimensional microscopy. *Biophys J*. 1999;77:2871–86. [https://doi.org/10.1016/S0006-3495\(99\)77119-5](https://doi.org/10.1016/S0006-3495(99)77119-5).
66. Iborra FJ, Pombo A, Jackson DA, Cook PR. Active RNA polymerases are localized within discrete transcription “factories” in human nuclei. *J Cell Sci*. 1996:1427–36.
67. Parada LA, McQueen PG, Misteli T. Tissue-specific spatial organization of genomes. *Genome Biol*. 2004;5:R44. <https://doi.org/10.1186/gb-2004-5-7-r44>.
68. Zeitz MJ, Mukherjee L, Bhattacharya S, Xu J, Berezney R. A probabilistic model for the arrangement of a subset of human chromosome territories in WI38 human fibroblasts. *J Cell Physiol*. 2009;221:120–9. <https://doi.org/10.1002/jcp.21842>.
69. Berrios S. Nuclear architecture of mouse spermatocytes: chromosome topology, heterochromatin, and nucleolus. *Cytogenet Genome Res*. 2017;151:61–71. <https://doi.org/10.1159/000460811>.
70. Berrios S, Fernández-Donoso R, Pincheira J, Page J, Manterola M, Cerda MC. Number and nuclear localisation of nucleoli in mammalian spermatocytes. *Genetica*. 2004;121:219–28. <https://doi.org/10.1023/B:GENE.0000039843.78522.99>.
71. Berrios S, Fernández-Donoso R. Nuclear architecture of human pachytene spermatocytes: quantitative analysis of associations between nucleolar and XY bivalents. *Hum Genet*. 1990;86:103–16. <https://doi.org/10.1007/BF00197690>.
72. Metzler-Guillemain C, Mignon C, Depetris D, Guichaoua MR, Mattei MG. Bivalent 15 regularly associates with the sex vesicle in normal male meiosis. *Chromosom Res*. 1999;7:369–78. <https://doi.org/10.1023/A:1009268014387>.
73. Codina-Pascual M, Navarro J, Oliver-Bonet M, Kraus J, Speicher MR, Arango O, et al. Behaviour of human heterochromatic regions during the synapsis of homologous chromosomes. *Hum Reprod*. 2006;21:1490–7. <https://doi.org/10.1093/humrep/del028>.
74. Sarrate Z, Blanco J, Vidal F. Acrocentric bivalents positioned preferentially nearby to the XY pair in metaphase I human spermatocytes. *Fertil Steril*. 2012;98:1241–5. <https://doi.org/10.1016/j.fertnstert.2012.07.1110>.
75. Burk RD, Szabo P, O’Brien S, Nash WG, Yu L, Smith KD. Organization and chromosomal specificity of autosomal homologs of human Y chromosome repeated DNA. *Chromosoma*. 1985;92:225–33. <https://doi.org/10.1007/BF00348698>.
76. Gardner RJ, Sutherland GR. Chromosome abnormalities and genetic counseling. 3rd ed. New York: Oxford University Press; 2004. [https://doi.org/10.1016/S1036-7314\(04\)80018-3](https://doi.org/10.1016/S1036-7314(04)80018-3).
77. Smith A, Fraser IS, Elliott G. An infertile male with balanced Y;19 translocation. Review of Y;autosome translocations. *Ann Genet*. 1979;22:189–94.

78. Vergés L, Blanco J, Valero O, Vidal F, Sarrate Z. Chromosome size, morphology, and gene density determine bivalent positioning in metaphase I human spermatocytes. *Fertil Steril*. 2014;101:818–24. <https://doi.org/10.1016/j.fertnstert.2013.11.013>.
79. Hazzouri M, Rousseaux S, Mongelard F, Usson Y, Pelletier R, Faure AK. Genome organization in the human sperm nucleus studied by FISH and confocal microscopy. Genome organization in the human sperm nucleus studied by FISH and confocal microscopy. *Mol Reprod Dev*. 2000;55:307–15. [https://doi.org/10.1002/\(SICI\)1098-2795\(200003\)55:3<307::AID-MRD9>3.0.CO;2-P](https://doi.org/10.1002/(SICI)1098-2795(200003)55:3<307::AID-MRD9>3.0.CO;2-P).
80. Zalenskaya IA, Zalensky AO. Non-random positioning of chromosomes in human sperm nuclei. *Chromosom Res*. 2004;12:163–73. <https://doi.org/10.1023/B:CHRO.0000013166.04629.97>.
81. Solov'eva L, Svetlova M, Bodinski D, Zalensky AO. Nature of telomere dimers and chromosome looping in human spermatozoa. *Chromosom Res*. 2004;12:817–23. <https://doi.org/10.1007/s10577-005-5513-1>.
82. Mudrak OS, Tomilin N, Zalensky AO. Chromosome architecture in the decondensing human sperm nucleus. *J Cell Sci*. 2005;118:4541–50. <https://doi.org/10.1242/jcs.02581>.
83. Manvelyan M, Hunstig F, Bhatt S, Mrasek K, Pellestor F, Weise A, et al. Chromosome distribution in human sperm - a 3D multi-color banding-study. *Mol Cytogenet*. 2008;1:25. <https://doi.org/10.1186/1755-8166-1-25>.
84. Ioannou D, Meershoek EJ, Christopikou D, Ellis M, Thornhill AR, Griffin DK. Nuclear organisation of sperm remains remarkably unaffected in the presence of defective spermatogenesis. *Chromosom Res*. 2011;19:741–53. <https://doi.org/10.1007/s10577-011-9238-z>.
85. Millan NM, Lau P, Hann M, Ioannou D, Hoffman D, Barrionuevo M, et al. Hierarchical radial and polar organisation of chromosomes in human sperm. *Chromosom Res*. 2012;20:875–87. <https://doi.org/10.1007/s10577-012-9323-y>.
86. Mudrak OS, Nazarov IB, Jones EL, Zalensky AO. Positioning of chromosomes in human spermatozoa is determined by ordered centromere arrangement. *PLoS One*. 2012;7:e52944. <https://doi.org/10.1371/journal.pone.0052944>.
87. Ioannou D, Millan NM, Jordan E, Tempest HG. A new model of sperm nuclear architecture following assessment of the organization of centromeres and telomeres in three-dimensions. *Sci Rep*. 2017;7:41585. <https://doi.org/10.1038/srep41585>.
88. Greaves IK, Rens W, Ferguson-Smith MA, Griffin D, Marshall Graves JA. Conservation of chromosome arrangement and position of the X in mammalian sperm suggests functional significance. *Chromosom Res*. 2003;11:503–12. <https://doi.org/10.1023/A:1024982929452>.
89. Foster HA, Abeydeera LR, Griffin DK, Bridger JM. Non-random chromosome positioning in mammalian sperm nuclei, with migration of the sex chromosomes during late spermatogenesis. *J Cell Sci*. 2005;118:1811–20. <https://doi.org/10.1242/jcs.02301>.
90. Zalensky AO, Allen MJ, Kobayashi A, Zalenskaya IA, Balhóm R, Bradbury EM. Well-defined genome architecture in the human sperm nucleus. *Chromosoma*. 1995;103:577–90. <https://doi.org/10.1007/BF00357684>.
91. Zalensky A, Zalenskaya I. Organization of chromosomes in spermatozoa: an additional layer of epigenetic information? *Biochem Soc Trans*. 2007;35:609–11. <https://doi.org/10.1042/BST0350609>.
92. Cremer T, Kurz A, Zirbel R, Dietzel S, Rinke B, Schröck E, et al. Role of chromosome territories in the functional compartmentalization of the cell nucleus. *Cold Spring Harb Symp Quant Biol*. 1993;58:777–92. <https://doi.org/10.1101/SQB.1993.058.01.085>.
93. Ni K, Spiess A-N, Schuppe H-C, Steger K. The impact of sperm protamine deficiency and sperm DNA damage on human male fertility: a systematic review and meta-analysis. *Andrology*. 2016;4:789–99. <https://doi.org/10.1111/andr.12216>.
94. Ferlin A, Raicu F, Gatta V, Zuccarello D, Palka G, Foresta C. Male infertility: role of genetic background. *Reprod BioMed Online*. 2007;14:734–45. [https://doi.org/10.1016/S1472-6483\(10\)60677-3](https://doi.org/10.1016/S1472-6483(10)60677-3).
95. Ioannou D, Tempest HG. Does genome organization matter in spermatozoa? A refined hypothesis to awaken the silent vessel. *Syst Biol Reprod Med*. 2018;2:1–17.
96. Hammadeh ME, Al-Hasani S, Stieber M, Rosenbaum P, Küpker D, Diedrich K, et al. The effect of chromatin condensation (aniline blue staining) and morphology (strict criteria) of human spermatozoa on fertilization, cleavage and pregnancy rates in an intracytoplasmic sperm injection programme. *Hum Reprod*. 1996;11:2468–71. <https://doi.org/10.1093/oxfordjournals.humrep.a019139>.
97. Depa-Martynow M, Kempisty B, Jagodziński PP, Pawelczyk L, Jedrzejczak P. Impact of protamine transcripts and their proteins on the quality and fertilization ability of sperm and the development of preimplantation embryos. *Reprod Biol*. 2012;12:57–72. [https://doi.org/10.1016/S1642-431X\(12\)60077-1](https://doi.org/10.1016/S1642-431X(12)60077-1).
98. Nasr-Esfahani MH, Razavi S, Mozdarani H, Mardani M, Azvagi H. Relationship between protamine deficiency with fertilization rate and incidence of sperm premature chromosomal condensation post-ICSI. *Andrologia*. 2004;36:95–100. <https://doi.org/10.1111/j.1439-0272.2004.00612.x>.
99. Nasr-Esfahani MH, Salehi M, Razavi S, Anjomshoa M, Rozbahani S, Moulavi F, et al. Effect of sperm DNA damage and sperm protamine deficiency on fertilization and embryo development post-ICSI. *Reprod BioMed Online*. 2005;11:198–205.
100. Iranpour FG. Impact of sperm chromatin evaluation on fertilization rate in intracytoplasmic sperm injection. *Adv Biomed Res*. 2014;3:229. <https://doi.org/10.4103/2277-9175.145719>.
101. Marchiani S, Tamburrino L, Benini F, Fanfani L, Dolce R, Rastrelli G, et al. Chromatin protamination and Catsper expression in spermatozoa predict clinical outcomes after assisted reproduction programs. *Sci Rep*. 2017;7:15122. <https://doi.org/10.1038/s41598-017-15351-3>.
102. Cremer T, Küpper K, Dietzel S, Fakan S. Higher order chromatin architecture in the cell nucleus: on the way from structure to function. *Biol Cell*. 2004;96:555–67.
103. Terada Y, Luetjens CM, Sutovsky P, Schatten G. Atypical decondensation of the sperm nucleus, delayed replication of the male genome, and sex chromosome positioning following intracytoplasmic human sperm injection (ICSI) into golden hamster eggs: does ICSI itself introduce chromosomal anomalies? *Fertil Steril*. 2000;74:454–60. [https://doi.org/10.1016/S0015-0282\(00\)00671-3](https://doi.org/10.1016/S0015-0282(00)00671-3).
104. Van Steirteghem A, Bonduelle M, Devroey P, Liebaers I. Follow-up of children born after ICSI. *Hum Reprod Update* 2002;8:111–116, DOI: <https://doi.org/10.1093/humupd/8.2.111>.