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Minireview

The Role of CCCTC-Binding Factor (CTCF) in Genomic Imprinting, Development, and Reproduction[1]

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ABSTRACT

CCCTC-binding factor (CTCF) is the major protein involved in insulator activity in vertebrates, with widespread DNA binding sites in the genome. CTCF participates in many processes related to global chromatin organization and remodeling, contributing to the repression or activation of gene transcription. It is also involved in epigenetic reprogramming and is essential during gametogenesis and embryo development. Abnormal DNA methylation patterns at CTCF motifs may impair CTCF binding to DNA, and are related to fertility disorders in mammals. Therefore, CTCF and its binding sites are important candidate regions to be investigated as molecular markers for gamete and embryo quality. This article reviews the role of CTCF in genomic imprinting, gametogenesis, and early embryo development and, moreover, highlights potential opportunities for environmental influences associated with assisted reproductive techniques (ARTs) to affect CTCF-mediated processes. We discuss the potential use of CTCF as a molecular marker for assessing gamete and embryo quality in the context of improving the efficiency and safety of ARTs.

assisted reproductive technology, chromatin, early development, epigenetics, genomic imprinting

INTRODUCTION

In recent decades, assisted reproductive techniques (ARTs), such as superovulation and embryo transfer (ET), in vitro embryo production (IVEP), intracytoplasmic sperm injection (ICSI), and cloning by somatic cell nuclear transfer (SCNT), have been developed for use in mammals [1]. Despite the great advances in ART protocols, in general, their efficiency remains low. Adverse effects of in vitro culture conditions on embryo quality have been described in the literature [2–8], and studies have shown that ARTs may induce imprinting errors [9–11]; however, the question has been raised as to whether the increased incidence of birth defects in ARTs is due to ARTs themselves or a consequence of fertility issues related to the parents [12–16]. Therefore, the consequences of adverse effects of ARTs on the health of animals and humans remain to be completely characterized. Among the environmental influences that may affect epigenetic factors, in vitro culture is one that has drawn attention [2, 3]. Studies have shown that culture medium, artificial atmosphere, and manipulation itself can have adverse effects on epigenetic profiles and gene expression patterns, and these can influence embryo quality [3–8, 17, 18]. This, in turn, may adversely affect fertility and the efficiency and safety of ARTs.

Widespread epigenetic reprogramming occurs at two stages of mammalian development: during gametogenesis and in early embryo development [19]. This process is followed by chromatin remodeling [20, 21]. While DNA methylation and histone modification patterns remain very stable through somatic cell divisions, during gametogenesis and initial embryo development, these patterns are highly susceptible to the influence of environmental and in vitro conditions [3, 17, 18, 22, 23]. Thus, the identification of epigenetic markers related to gamete and embryo quality is necessary to support the development and optimization of new protocols that increase the efficiency and safety of ARTs in humans and animals. With this goal, the majority of studies performed up to now have been focused on gene expression and DNA methylation. Considering that epigenetic mechanisms involve DNA methylation and posttranslational histone modifications, influencing other proteins that bind to DNA, studies addressing all these aspects and the DNA-protein binding patterns are essential to understanding how chromatin regulates gene expression. The CCCTC-binding factor (CTCF) is an important candidate molecular marker, because it is the main known protein required for insulator activity in vertebrates [24], it has genome-wide, methylation-sensitive binding sites [25–27], and it participates in imprinting establishment and global gene regulation, activating or repressing gene expression.

The role of CTCF in genomic imprinting, gametogenesis, the early stages of mammalian development, and fertility are considered along with the potential adverse effects of ARTs on CTCF function. Also discussed are perspectives for CTCF as a molecular marker to assess gamete and embryo quality in order to improve the efficiency of ARTs.

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CTCF STRUCTURE AND FUNCTION

CTCF is an 11-zinc finger (ZF) protein flanked by unstructured N- and C-terminal polypeptides, containing ~727 amino acids and two transcription repressor domains, with flexible binding to divergent regulatory DNA sequences [28–30]. CTCF domains are highly conserved among vertebrates [31, 32], consistent with an essential role for CTCF in cellular function [27]. CTCF is an evolutionarily conserved protein that performs similar functions at dissimilar sequences [33], although most of its genomic targets carry a conserved consensus core motif [34, 35]. Despite the presence of 11 ZF domains in the CTCF protein sequence, only four of them are necessary to strongly bind to DNA, recognizing a core 12-bp DNA sequence [34, 36].

Binding of CTCF to DNA was first identified at the 5′-flanking CTC-rich DNA sequences of the *CMYC* gene, and it was termed the ''CCCTC-binding factor'' [29, 37]. However, empirical studies reveal the presence of the canonical CCCTC motif alone is neither necessary nor sufficient for CTCF binding to DNA in vivo and in vitro [38]. CTCF is able to mediate the formation of DNA loops [39], and its ability to induce chromosomal loops enables it to regulate gene function over long distances in the genome [40]. Furthermore, it interacts with the nuclear lamina, which is a protein network, participating in chromatin-nuclear membrane attachments in a highly complex nuclear organization that can drive global genome function [41]. Alongside chromosome looping, CTCF is involved in nucleosome positioning, supporting another essential chromatin remodeling process [42, 43]. Using genome-wide mapping, Kim et al. [27] identified ~14 000 CTCF-bound sites across the genome in human fibroblasts, and Prickett et al. [25] identified ~50 000 CTCF-bound sites in mouse brain, consistent with a role for CTCF in whole-genome chromatin organization. Li et al. [26] evaluated CTCF binding in 56 human cell lines from the ENCODE project and identified 23 709 constitutive CTCF-binding sites that were occupied in more than 90% of cell lines, implicating CTCF in fundamental biological processes.

Consistent with a genome-wide role in gene regulation, CTCF can associate with a variety of different factors on DNA and chromatin, performing many functions using different partners [44]. For example, it interacts with a Y-box DNA/RNA binding factor, YB1, that is involved in a range of cellular processes, including transcription, DNA replication, and RNA processing [38]. Although the HDAC1 and HDAC2 enzymes are components of the nuclear matrix, the insulator activity of CTCF seems to be in part related to its ability to interact with nuclear matrix proteins using an HDAC-independent mechanism [45]. CTCF interacts with kaiso, a member of the POZ (pox virus and ZF) family of ZF transcription factors, where there are kaiso binding sites at CTCF target genes, suggesting negative regulation of CTCF insulator activity [46]. In addition, coincident binding of CTCF and cohesin complex is reported genome wide [25, 47, 48], participating in the establishment of DNA loops involved in gene activation or repression, depending on the context of the tissue-specific chromatin organization [48]. CTCF binds cohesin via direct contact between the cohesin SA2 subunit and its C-terminal tail [49], mediating both transcriptional insulation [50] and sister chromatid cohesion during cell division [51]. Despite the genome-wide association between CTCF and cohesin, it seems that both contribute differently to chromatin architecture and gene transcription [52]. Alongside a role in mediating chromosome structure and remodeling, association between CTCF and RNA polymerase II in vivo and in vitro suggests a direct participation in gene transcription [53] and alternative splicing [54]. CTCF function can be regulated by posttranslational modifications, such as poly (ADP-ribosyl)ation [55, 56] and by the small ubiquitin-like protein (SUMO), which conjugates with CTCF, inducing repressive chromatin organization and consequently reducing CTCF transcriptional activity [57, 58]. Figure 1 provides a summary of the interactions between CTCF and some of its partners, and the effects of these interactions on chromatin are shown.

CTCF, DNA METHYLATION, AND GENOMIC IMPRINTING

DNA methylation is generally considered a repressive mark, related to gene silencing when it is located at the promoters of genes. In mammals, DNA methylation mostly occurs at the cytosine of CpG dinucleotides, which are unevenly distributed throughout the genome. Gene promoters and intragenic regions contain short sequences of ~250–3500 bp with a high G + C content known as CpG islands [59], which are frequently unmethylated. Conversely, in the bulk of the genome, the CpG dinucleotide is depleted because of deamination [60], and usually methylated, although a variation in the methylation pattern can be observed among different tissues [61]. CTCF binds to DNA in a methylation-sensitive manner [54, 62], because methylation at specific CpG sites in selected CTCF-binding motifs influences binding efficiency [36]. Furthermore, CTCF may be involved in the maintenance of the unmethylated DNA; for example, after abolishing CTCF binding at the human retinoblastoma (*RB*) gene promoter, a progressive gain of methylation has been observed [63]. Reinforcing this, CTCF can complex with DNMT1 and PARylated PARP1 (poly [ADP-ribose] polymerase 1), which maintain unmethylated CTCF-bound CpGs [64] (Fig. 1). However, it remains to be completely clarified if unmethylated sequences facilitate CTCF binding, or if bound CTCF maintains unmethylated DNA.

Imprinted genes are expressed in a parent-of-origin-specific manner controlled by allele-specific DNA methylation marks established in the gametes. They are frequently organized in clusters in the genome, and several imprinted clusters harbor CTCF-dependent insulators involved in the regulation of monoallelic expression [65]. *Igf2* is an important fetal mitogen gene involved in normal embryo development. After fertilization, the precise control of *Igf2* expression is essential for normal embryo and fetal development, and the *H19*/*Igf2* locus can be susceptible to in vitro culture [66]. This locus is one of the best-understood models of imprinted gene regulation, and CTCF participates centrally in its regulation. Due to reduced affinity for methylated DNA, CTCF colocalizes with cohesin [67] and binds to the unmethylated maternal allele at the *H19*/*IGF2* imprinted control region (ICR), which is also enriched for H3K4 methylation [9], inducing intrachromosomal loop formation [68] and recruitment of the polycomb repressive complex 2 (PRC2) through CTCF-SUZ12 interactions. This leads to methylation of H3K27 and suppression of *Igf2*/*IGF2* expression from the maternal allele [69, 70]. On the other hand, the paternal ICR is highly methylated and enriched for H3K9 methylation, preventing CTCF/cohesin complex binding and contributing to *Igf2* expression [9, 68]. Vigilin is a new CTCF partner that has recently been identified to colocalize with CTCF, further contributing to regulation of the *H19*/*IGF2* locus [71]. The model of regulation at the *H19*/*IGF2* imprinted locus is shown in Figure 2, in which some of the main known events involving CTCF are summarized. Monoallelically expressed genes, including imprinted genes, are asynchronous-

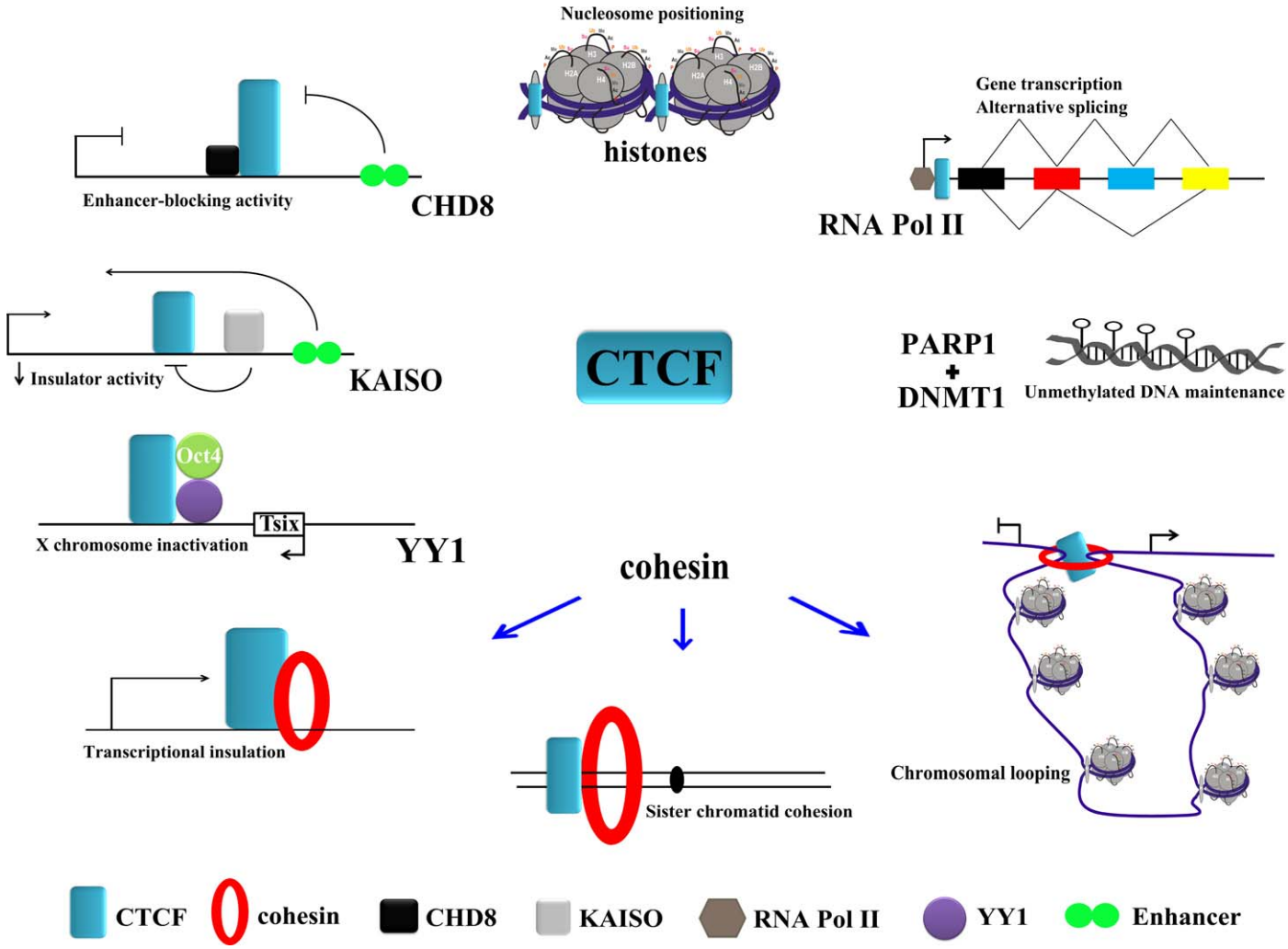

FIG. 1. Summary of interactions between CTCF and some of its partners, showing the different functions performed as a result of these interactions within the genome.

ly replicated during S phase of the cell cycle [72, 73]. Consistent with this, loss of CTCF binding at the *H19*/*Igf2* ICR results in loss of asynchronous replication [72]. Knockdown of SNF2-like chromodomain helicase protein CHD8 [74] disrupts the *H19* DMR and reactivates the maternal allele of *IGF2*, suggesting that the enhancer-blocking activity depends on a CHD8-CTCF interaction [74] (Fig. 1). Introduction of point mutations in each of the four CTCF DNA-binding motifs at the mouse *H19* ICR ablates CTCF binding and leads to activation *Igf2* on the maternal allele [75]. Mutation of only CTCF site 4 in the *H19* ICR leads to DNA methylation and activation of the maternal *Igf2* allele, so that the methylation-free domain of the maternal allele requires the cooperative participation of all four CTCF sites [76]. In addition, expression levels of the *CTCF* gene may also be involved in the imprinting control of the *IGF2* locus. A complete loss of *IGF2* imprinting, with biallelic expression and increased *IGF2* expression in senescent human cells, is correlated with a decrease in CTCF binding due to reduced *CTCF* expression [77]. Other relatively well-characterized imprinted loci include *Dlk1-Dio3* and *Kcnq1*/*Kcnq1ot1* [65, 78]. CTCF colocalizes with cohesin, showing distinct binding at the *Gtl2* and *Kcnq1ot1* DMRs, likely indicating locus-specific functions in the control of imprinted genes [78].

CTCF, GAMETOGENESIS, AND EMBRYO DEVELOPMENT

During mammalian embryo development, primordial germ cells (PGCs), originating from the inner cell mass and precursors of the gametes migrate to colonize the initial gonads. In the early stage of gametogenesis, PGCs undergo genome-wide demethylation, followed by the establishment of a sex-specific pattern of epigenetic programming in the gametes [19]. As a consequence of this, mature oocytes and spermatozoa show a sex-specific chromatin configuration during the fertilization period. After fertilization, a new wave of demethylation and histone modification occurs, culminating with the development of embryonic stem cells (ESCs), which are epigenetically able to give rise to all the different tissues of an organism. Despite many studies evaluating DNA methylation reprogramming in gametes and embryos, the role of CTCF in this window of development is relatively unclear. When maternal stores of CTCF are depleted from mouse oocytes using a transgenic RNAi strategy, hundreds of genes are misregulated, suggesting an essential role of CTCF during oogenesis and embryo development [24]. CTCF may activate or derepress transcription in oocytes, and its depletion can induce mitotic defects and apoptosis [24]. Moreover, in the presence of maternal CTCF, zygotic CTCF expression does not

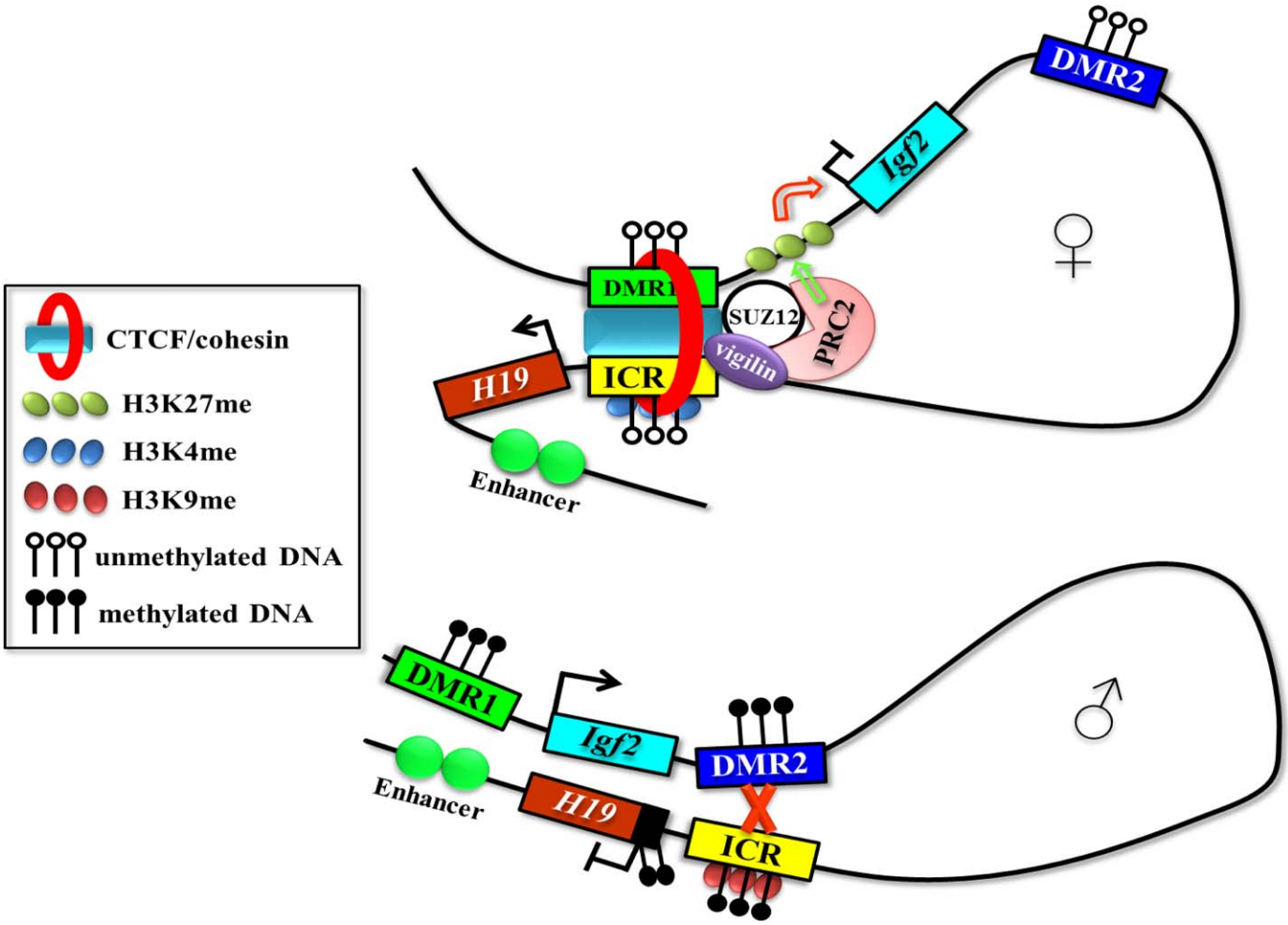

FIG. 2. Model of regulation at the imprinted *H19/Igf2* locus, showing the role of CTCF in this process. In the maternal chromosome, the CTCF/cohesin complex binds to the unmethylated ICR, which is enriched for methylation at H3K4, and to the unmethylated *Igf2* DMR1. Consequently, a specific intrachromosomal loop is induced; the SUZ12 subunit attracts the PRC2 complex, which induces methylation at H3K27, repressing *Igf2* expression. Additionally, vigilin, which is a recently identified CTCF partner, also binds to the CTCF complex on the active maternal allele. In the paternal chromosome, the ICR is methylated and enriched for methylation at H3K9, and CTCF is unable to bind. The methylated ICR links to the methylated *Igf2* DMR2, inducing a different intrachromosomal loop formation, allowing enhancers to access *Igf2*, which is expressed, whereas *H19* is silenced.

seem to be required for preimplantation mouse embryo development, and the loss of maternal transcripts is associated with apoptosis in the developing embryo and peri-implantation lethality [79]. During mouse ESC differentiation, unmethylated CpG islands showed reduced nucleosome occupancy and enrichment in CTCF-binding sites [80]. Additionally, CTCF binds at or near many pluripotency genes, and is critical for human ESC proliferation [81]. During oocyte growth, hypomethylated oocytes gain a sex-specific pattern of DNA methylation. Schoenherr et al. [75], using mutant mice, showed that CTCF binding is not necessary to establish the unmethylated status of the *H19* ICR during oogenesis, suggesting that a factor other than CTCF may protect the ICR from methylation during oogenesis. On the other hand, Fedoriw et al. [82] and Engel et al. [83] have suggested that CTCF protects the *H19* DMD from de novo methylation during oocyte growth and after implantation, respectively. Despite these conflicting results, it is clear that CTCF plays an important role in the identity of the maternal *H19* allele during oogenesis [84]. In addition, replacing the *H19/Igf2* ICR with two copies of the chicken β-globin insulator, which does not became methylated in male germ line, resulted in biallelic insulation by CTCF of the *H19/Igf2* locus and, subsequently, fetal growth retardation and late fetal/perinatal lethality [85].

One important event that occurs during mammalian embryo development is X chromosome inactivation (XCI), a process that has evolved to equalize the expression levels of X-linked genes between males and females [86]. Many factors and specific epigenetic marks are involved in XCI, and CTCF is essential for the *cis*-acting choice of which parental X chromosome will remain active, interacting with YY1, OCT4 [87–89], the *Jpx* noncoding RNA [90], and other factors. X chromosome pairing is an important event in the initiation of the XCI process [91]. CTCF is implicated in this event, in agreement with its function in mediating long-range chromatin interactions [91], and depletion of CTCF results in aberrant XCI [89, 92]. CTCF interacts with the *XIST/Xist* promoter in female human and mouse cells, and specific point mutations in the promoter sequence reduce CTCF binding affinity, skewing the choice of which X will be inactivated [93]. Therefore, altered expression and/or mutations of CTCF-binding sites may disrupt XCI, blocking the developmental process in mammalian female embryos.

The paralogous gene to mammalian *CTCF/Ctcf*, *BORIS/Boris*, encodes the same 11-ZF domain and, thus, has the same

DNA binding potential, but the N-terminal sequence does not exhibit significant homology [94], suggesting interactions with different proteins. *BORIS*/*Boris* is present in testis [94, 95], indicating a likely role in male germ line establishment [96] and absence of expression results in subfertility [97]. Knockout mice have defects in spermatogenesis with dramatically reduced expression of the cerebroside sulfotransferase (*Cst*) gene, which is known to play a crucial role in meiosis [98]. During mouse spermatogenesis, the paternal-specific methylation pattern is acquired by the maternal *H19* allele concomitantly with activation of *Boris*, and this allele is largely unmethylated while interacting with BORIS [99]. Moreover, in the prospermatogonial stage, both unmethylated *H19*/*Igf2* alleles show different rates of de novo methylation, with the maternal allele presenting a CTCF-dependent delay in gain of methylation [100]. Unlike oocyte chromatin, the haploid nucleosomal chromatin of sperm is repackaged, exchanging histones for small basic protamines, and thus generating highly compact toroidal chromatin essential to sperm function [101, 102]. Despite this change, a small proportion of sperm chromatin maintains its nucleosomal structure [103–105]. This structure is important to zygotic chromatin, involved in the paternal epigenetic reprogramming after fertilization [106], related to embryo development and the establishment of embryonic totipotency [21], and it is closely associated with gene promoters and sequences recognized by CTCF [107, 108]. The in vivo sperm capacitation process, which is acquired in the female reproductive tract and enables spermatozoa to fertilize an oocyte, is accompanied by an increase in tyrosine phosphorylation of a subset of proteins. A capacitation-related tyrosine phosphorylation of serine/threonine-phosphorylated CTCF occurs, enhancing the affinity of the capacitated sperm CTCF to its target DNA [109]. Taken together, this links CTCF with the chromatin configuration that is essential for initial stages of embryo development after fertilization and totipotency.

CTCF, FERTILITY, AND ARTS

ARTs, such as superovulation and ET, ovum pickup, and IVEP, ICSI, and cloning by SCNT are used worldwide in animals [1], contributing to animal breeding and animal conservation programs. In 2011, 1 230 773 in vivo and in vitro embryos were produced worldwide in domestic animals (IETS, International Embryo Transfer Society [http://www.iets.org/]) [110]. In humans, since the first IVEP baby was born in 1978, an estimated 5 million such babies have been born worldwide (ESHRE, European Society of Human Reproduction and Embriology [http://www.eshre.eu]) [111], enabling couples with fertility problems to have children. Despite the routine use and significant advance of ART protocols in the last decades, their efficiency remains low, with in vitro culture conditions adversely affecting gamete and embryo quality.

In vitro maturation of oocytes may interfere with imprinting establishment, altering gene expression [112, 113], and impairing oocytes and preventing them from generating good-quality embryos. Furthermore, DNA methylation alterations are associated with subfertility and/or infertility in humans and animals, and abnormal DNA methylation at the CTCF-binding site 6 of the *H19* DMR is closely associated with oligozoospermia [114, 115], azoospermia [116], teratozoospermia, and oligo-astheno-teratozoospermia [117] in humans, and altered conception rates in cattle [118]. Interestingly, the same sixth CTCF-binding site was found to be hypomethylated in placentas of pregnancies conceived by IVEP/ICSI [119]. Whether the altered DNA methylation of the CTCF-binding site 6 is due to the IVEP/ICSI techniques or is a result of parental fertility problems remains to be clarified, considering that both in vitro manipulations and infertility may be correlated with epigenetic abnormalities. The methylation status of the *H19* DMR seems to be susceptible to in vitro culture conditions, with CTCF-binding sites revealing an aberrantly methylated state in MI and MII human oocytes [112]. However, it would be useful to know whether this altered methylation pattern impairs CTCF binding to DNA and influences oocyte competence. There is documented evidence that CTCF is unable to link to the *H19* methylated allele [84].

IVEP induces abnormal DNA methylation and histone modifications at the *H19*/*Igf2* CTCF-binding site [9], and perturbations of *H19*/*IGF2* and *KvDMR1* loci, mainly in ART-conceived individuals, are related to two similar overgrowth syndromes in humans and animals, Beckwith-Wiedemann syndrome (BWS) and large offspring syndrome, respectively [120–123]. Microdeletions abolishing CTCF target sites on the *H19* DMR locus are related to BWS in humans [120, 121]. In pigs, CTCF-binding site 3 showed abnormal methylation in cloned offspring, which may contribute to the low efficiency of porcine SCNT [10]. In bovine cloned embryos, demethylation of the CTCF site on the paternal *H19* DMR was found, suggesting that this alteration could be associated with reduced size and low implantation rates of cloned embryos in cattle [11]. In addition, altered imprinting control with abnormal hypomethylation of the *KvDMR1* domain on bovine chromosome 29 and altered gene expression in cloned and IVEP-produced calves have been found [122].

Considering the adverse influence of environment on development and fertility, exposure of pregnant mice to ethanol alters the methylation status of CTCF-binding sites in imprinted loci in the brain [124] and decreases the methylation status of CpGs in CTCF-binding site 2 of the *H19* DMR and sperm concentration in the offspring [23]. Likewise, preconception paternal alcohol exposure decreased the methylation status of CTCF-binding sites 1 and 2 in offspring and reduced postnatal offspring weight [125].

Mutations in the *CTCF* gene may correlate with developmental disorders in humans and animals. Although no mutations have been found in the coding sequence of the *CTCF* gene in BWS patients who show gain of methylation in the *H19*/*IGF2* ICR [126], mutations in *CTCF* have been found in individuals with intellectual disability, microcephaly, and growth retardation [127]. Additionally, these individuals show deregulation of genes involved in signal transduction, which may affect developmental processes and cognition, suggesting that CTCF has a role in driving gene regulation and development [127]. Furthermore, a single-nucleotide polymorphism (SNP) in the 5′ region of the *H19* gene and located near CTCF-binding sites has been associated with abnormal birth weight in humans, with a decrease or increase in birth weight, depending on the parental origin of allele [128]; furthermore, SNPs located in critical CTCF and OCT4 binding sites of the *H19* ICR region have been associated with increased hypermethylation of specific CpG sites of the maternal *H19* allele [129, 130] in BWS patients [130], which, in turn, may affect CTCF binding and subsequent *IGF2* expression and growth. Additionally, SNPs in another imprinted gene, *KCNQ1*, affect CTCF binding to DNA and also correlate with BWS risk [131]. CTCF is also involved, in a methylation-dependent manner, in the modulation of genetic repeat instability, which, in turn, is related to several human disorders resulting from microsatellite expansion [132].

While the role of CTCF in cellular differentiation is not yet fully clear, it is involved in mouse and zebrafish myogenesis

and development, and is indispensable for muscle development [133]. In this sense, considering that many developmental disorders and fetal weight problems that occur in SCNT cloning are also linked to placental alterations [134, 135], abortions [1, 136, 137], and other alterations in early life [138], we speculate that CTCF may be involved in these disorders, possibly through mechanisms associated with the low efficiency of the technique. Furthermore, CTCF involvement may be related to the deregulated DNA methylation reprogramming that is generally found in SCNT [17], considering that CTCF may bind DNA in a methylation-sensitive manner. The strength of evidence reviewed here suggests that *CTCF* and its DNA binding sites are important candidate regions to be investigated as molecular markers involved in fertility, growth, and developmental disorders related to environmental effects on the genome and ARTs. To provide evidence for this, evaluation of the methylation status, point mutations, and CTCF occupancy at the *H19*/*IGF2* CTCF-binding region in the context of ART may reveal this region as a novel candidate molecular marker in ARTs.

CONCLUSIONS

In recent decades, the use of ARTs has increased enormously. Nevertheless, their efficiency remains low, demanding, for example, the transference of multiple human embryos to the uterus to obtain pregnancies, which increases twin birth rates. Furthermore, two of the most important topics that remain to be elucidated are the understanding of the adverse influence of in vitro culture and of the fertility problems of the parents on the health of ART-conceived individuals, especially the long-term effects manifested in adulthood. The use of the highest quality gametes to produce embryos in the context of ARTs is one way to overcome these problems. More targeted strategies are needed to improve IVEP protocols. The identification of molecular markers associated with gamete and embryo quality is essential to ensure the identification of the best gametes and embryos to be used and the development and optimization of new IVEP protocols with increased efficiency and safety. CTCF may be a good candidate molecular marker due to its participation in gamete and embryo chromatin configuration, imprinting, and gene regulation. It is established that the methylation patterns of CTCF sites influence imprinting, fertility, and the efficiency of ARTs. However, the majority of studies have focused only on one specific locus, and the mechanisms of how methylation patterns influence phenotypes are not completely clear. Therefore, it is also important to evaluate other imprinted and nonimprinted domains in the genome. All of the molecular mechanisms that affect the capacity of CTCF binding to DNA are relevant to investigate, considering the importance of this major insulator protein on chromatin and gene regulation genome wide. Studies that evaluate the genome-wide DNA methylation and mutations of CTCF sites and the mutations and expression levels of the *CTCF* gene are investigations of important strategies aimed at establishing CTCF as a molecular marker in reproductive genetics. However, obtaining the necessary quantities of chromatin and/or DNA, especially from human oocytes and embryos, required for the techniques used in genome-wide evaluations still remains a challenge. The development of noninvasive molecular tools for the assessment of molecular markers, such as cumulus cell biopsies and low-cell number ChIP-seq, will allow researchers to develop new methods to assess and screen ART oocytes and embryos.

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