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CRISPR In the Field of Assisted Reproductive Technology (ART)

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ABSTRACT:

Technology for Assisted Reproduction (ART) has transformed fertility treatments, offering hope. globally. Techniques like Intra Cytoplasmic Sperm Injection (ICSI) and In Vitro Fertilization (IVF) manipulate eggs, sperm, or embryos, increasing successful pregnancy. CRISPR, a revolutionary RNA-guided tool, derived from bacteria's defence mechanism against infections, has sparked interest in ART. CRISPR/Cas9, composed of two parts: CRISPR-Associated Protein 9 (Cas9) and Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR) that shows promise in treating diseases by enabling precise genome editing. At early stages, the genetic material of sperm and oocyte cells is still distinct; later on, however, genetic material combines and, as a result of varying gene expression, forms diverse shapes. To employ CRISPR/Cas9 for gene editing in humans, embryos must be safely cultivated through IVF to ensure optimal development. It integrates with Preimplantation Genetic Testing (PGT), identifying monogenic diseases in embryos or gametes, allowing for accurate treatment and unforeseen effects detection, enhancing gene editing in human health. This paper provides a review of the role of CRISPR in the field of ART, exploring its potential applications, ethical considerations, and future implications. We discuss how CRISPR enables a precise genome. editing, offering unprecedented opportunities for addressing genetic disorders, enhancing fertility outcomes, and advancing our understanding of reproductive biology. Furthermore, we examine the ethical dilemmas surrounding the use of CRISPR in reproductive contexts, including concerns about safety, equity, and the potential for unintended consequences. Finally, highlight the need for interdisciplinary collaboration, robust regulations, and ongoing dialogue to ensure ethical and responsible use of CRISPR in assisted reproduction.

KEYWORDS*: Assisted Reproductive Technology (ART), In Vitro Fertilization (IVF), Intra Cytoplasmic Sperm Injection (ICSI), CRISPR-Cas9, RNA-guided tool, bacteria's defence mechanism, genome editing, fertility treatments Preimplantation Genetic Testing (PGT), monogenic diseases, ethical considerations, interdisciplinary collaboration, robust regulations.*

INTRODUCTION:

Assisted reproductive technology (ART) refers to fertility treatments and procedures that can help with difficulties or an inability to conceive children. ART techniques involve the manipulation of eggs, sperm, or embryos to increase the likelihood of a successful pregnancy. Procedures including in vitro fertilisation (IVF), intracytoplasmic sperm injection (ICSI), gamete or embryo cryopreservation, and/or the use of fertility medications are included. Apart from this parents at risk of transmitting genetic conditions to their offspring can also seek to procreate with the aid of assisted reproductive technologies (ARTs) and preimplantation screening technologies (such as PGD), which would allow them to have genetically related children free from the condition that affects them (Cavaliere, 2018). With PGD, it is possible to screen IVF-created embryos for genetic defects before transferring them into the uterus.

Early in 2015, a group of Chinese scientists used a novel gene editing technology on human embryos that were not viable, which caused controversy (David et al, 2015). Clustered regularly interspaced short palindromic repeats, or CRISPR, and CRISPR-associated protein 9 (Cas9) are the two components of the RNA-guided CRISPR/Cas9 technology. Using a naturally occurring defense mechanism, CRISPR/Cas9 allows bacteria to evade damaging infections brought on by pathogenic species, such as viruses. CRISPR serves as an RNA tool that directs the Cas proteins to target particular regions of the genome, which the Cas proteins then cut. These broken strands can be used to introduce genes at the cut spot and change the DNA's nucleotide sequence. The use of this method on human embryos and gametes—that is, oocytes and sperm cells—has drawn a lot of criticism for a variety of reasons, most notably the possibility that it could result in heritable alterations to the human genome (germline modification).

The three stages of CRISPR/Cas9 editing involve recognition, cleavage, and repair of genomic material. The technique uses complementary base pairing to identify the target gene sequence via guide RNA (gRNA). Next, three base pairs up from the protospacer adjacent motif—a short DNA sequence adjacent to the cleavage site—the Cas9 nuclease causes double strand breaks, which causes local DNA melting. Then, this break is fixed using either homology-directed repair (HDR), in which homologous donor DNA fixes the DNA damage, or non-homologous end joining, in which damaged ends of DNA are brought together. (Manne, 2023).

Germline Gene Editing (GGE)

To assess the viability of germline gene editing (GGE) utilising CRISPR/Cas9, it is necessary to comprehend the multitude of systems and procedures that are involved. Understanding the complex stages of embryonic development is a prerequisite for putting this technology into practice. In sperm and oocyte cells, genetic material is still distinct in the early stages. However, in the later stages, genetic material combines and, as a result of variable gene expression, forms diverse shapes. Due to its alien nature, access to genetic material at different phases of embryonic development may require different defence systems or complicate the usage of CRISPR/Cas9. Knowing more about embryonic development will help determine whether the CRISPR process has any undesirable or aberrant impacts (Manne, 2023).

To employ CRISPR/Cas9 for gene editing in humans, embryos must be safely cultivated through in vitro fertilization (IVF) to ensure optimal development. While attempts at germline genome editing (GGE) have been made in vivo in mammals (Kalebic et al., 2016) (Abu-Bonsrah et al., 2016) the more regulated and ethically sensitive context of human GGE necessitates the controlled environment of IVF. Establishing a very effective and secure IVF process for fertilisation, embryo selection, and gamete analysis becomes a crucial initial step. Moreover, the competitive IVF market stands to gain increased effectiveness via the utilization of CRISPR. An integral aspect utilising this gene-editing tool involves identifying sequences to edit and screening to confirm accurate edits post-procedure. With over 5000-6000 known monogenic phenotypes, CRISPR/Cas9 holds significant potential for treating various diseases (Rodwell et al., 2014). Preimplantation genetic testing (PGT) allows identification of these monogenic diseases in embryos or parental gametes before treatment. Post-treatment, CRISPR/Cas9 facilitates the detection of treatment accuracy and unforeseen effects, providing a comprehensive approach towards human health and gene editing.

The system CRISPR, well-known for its efficiency and cost-effectiveness in genome editing, has gained increased attention. CRISPR/Cas9 facilitates the correction of gene mutations, alteration of gene expression through epigenetic manipulation, and direct visualization of proteins, addressing significant scientific inquiries and aiding in disease treatment across human and other species. CRISPR has been the main method used in the past ten years by people who want to change genes, both human and non-human. It has been used in trials to create malaria-resistant insects, genetically change disease-resistant plants, investigate the prospect of creating animals and pets that are altered, and maybe treat a few human diseases (such as HIV, hemophilia, and leukemia).However, grappling with challenges such as, HDR rate, on-target effects and off-target effects poses a considerable hurdle for the CRISPR/Cas9 technique. Despite ongoing enhancements to CRISPR systems, previously overlooked errors in gene editing continue to emerge, and oversights in genome editing may introduce uncertainties regarding offspring health. Addressing these limitations and associated ethical concerns is imperative before extending the application of this technology in mammalian embryos and oocytes. (Zhang et al., 2023).

2.REVIEW OF LITERATURE:

2.1: CRISPR and its Mechanism of Action..

Less than ten years ago, the CRISPR system's potential for genome editing skyrocketed, earning Jennifer Doudna and Emmanuelle Charpentier the 2020 Chemistry Nobel Prize. Bacteria and Archaea use the CRISPR system to protect themselves against viral infections. Part of the phage genome may be integrated into a particular locus known as CRISPR, which is made up of short DNA repeats (length: 23–55 base pairs, bp) (Barrangou and Marraffini, 2014), turning it into a CRISPR DNA spacer (length: 21–72 bp), if the host survives the initial viral onslaught. The bacterial genome contains various loci for CRISPR, and each locus has the potential to contain multiple spacers (Haliangium ochraceum has up to 600 spacers). "Adaptation" is the term for this stage of the procedure.The CRISPR locus, which typically comprises 50 spacers per CRISPR array (Barrangou and Marraffini, 2014), is translated into a long RNA (CRISPR RNA, or crRNA), which is cleaved into short interfering crRNA if the bacterium becomes infected with the same type of virus again. These fragments will attach to the freshly injected viral genome along with the Cas protein and tracrRNA, causing a nucleolytic cut that will promote the virus's degradation—a process referred to as "interference." The Cas (CRISPR-associated protein) family of proteins contains the protein or proteins that are responsible for the pairing and cut. Different CRISPR systems have different targets for nucleic acids, such DNA or RNA, and they have different structural characteristics, like single- or double-stranded, linear or circular DNA. These systems fall into two groups, which are then separated into 33 subtypes and six types. Class II systems use a single, bigger Cas protein, such as the widely used Cas9 endonuclease, for DNA degradation, whereas Class I systems use numerous Cas proteins. (Piergentili et al., 2021).
Class II includes the well-known genome editing tool Cas9 complex. It consists of the Cas9 protein, which contains the DNA-cutting HNH and RuvC endonuclease domains, and two RNA molecules: trans-activating CRISPR RNA (tracrRNA) and crRNA. The tracrRNA, which is known as "guide RNA" (gRNA), forms a duplex that directs the complex to its target and is essential for crRNA maturation. This complex is processed by RNAse III, which guarantees accurate target recognition. In Cas9-mediated target degradation, the Protospacer Adjacent Motif (PAM) sequence is a crucial component. PAM is a brief segment of DNA on the non-target strand that is next to the cleavage point. In order to differentiate self from non-self DNA and avoid making mistakes while cutting bacterial DNA at the CRISPR locus, it is necessary for the crRNA sequence to have this region.This recognition mechanism is vital for precise DNA targeting in genome engineering. (Piergentili et al., 2021).
The goal of Doudna and Charpentier's research was to improve the CRISPR/Cas9 system so that it could be used to specifically alter genomic sequences in a variety of animals. By fusing two RNA molecules into a synthetic single guide RNA (sgRNA), they converted the Cas9 complex into a regulated, two-component system. Depending on the repair mechanism at work, this sgRNA directs the Cas9 endonuclease to the appropriate DNA target, allowing for either gene deletion or knockin. The DNA is cut at the identified target sequence by the Cas9 endonuclease with the introduction of a specially designed sgRNA in vitro. This triggers the DNA repair machinery of the host cell, which uses the clumsy NHEJ method for gene knockout. As an alternative, homology-driven DNA repair can take place in the presence of an exogenous template (donor DNA).This makes it possible to replace the original sequence with a desired one, enabling particular mutations, fixing mutated genes, or adding whole new genes. The amount of the donor DNA might vary, ranging from huge sections carrying numerous genes to a few base pairs for gene point mutations. Due to its simplicity of usage and ability to precisely target DNA regions, this method of changing genomic DNA has been widely adopted in a variety of living creatures, including humans, animals, and plants (Piergentili et al., 2021).

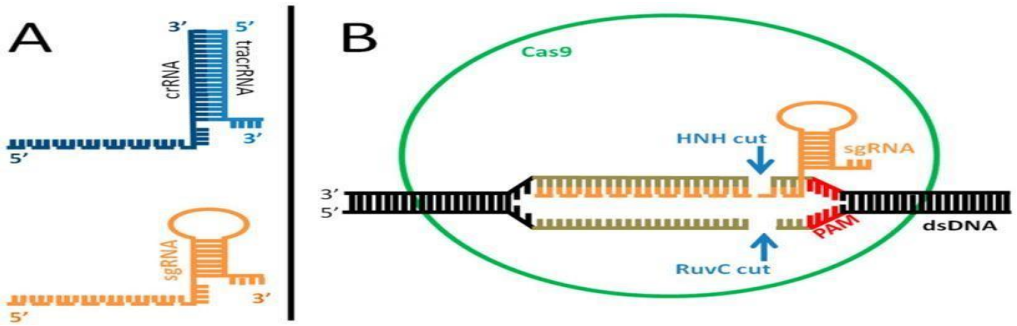

Fig 1: A) The Cas complex operates through a natural or engineered guide RNA (gRNA). In the natural system, crRNA and tracrRNA form two parts, guiding Cas9 to target viral DNA.. The sgRNA is integrated into the Cas9 protein, which mimics the natural structure for genome editing. It recognizes the double-stranded target DNA and promotes pairing. When the target DNA has a PAM sequence (red) and homology (21–72 bp sequence length, shown in gray), the Cas protein cuts 3 bp upstream of PAM, resulting in a double-strand break and inactivating the target gene. In most eukaryotic cells, the error-prone NHEJ process is used for repair. However, the cell may use homology-driven repair to introduce the desired sequence into the genome when an exogenous DNA template is present(Piergentili et al., 2021).

2.2: New Area Of Research.

There has been a lot of continuing research in this area to understand the effects, opportunities for improvement, uses and related ethical considerations on human embryos of the gene editing system, in addition to crucial advancements in IVF and PGT technologies that will enable CRISPR/Cas9 for GGE.

Chinese researchers initially applied Germline Genome Editing (GGE) in 2015, leading to the birth of the first human twins with altered DNA in 2018. To produce HIV-resistant offspring, He Jiankui and his colleagues at the Southern University of Science and Technology in Shenzhen disabled the CCR5 gene using CRISPR/Cas9. (Regalado, 2018). Although the CCR5 gene is essential for many important brain functions, worries were raised despite the planned benefits because the possible impacts on the twins were not sufficiently addressed.

Since these experiments, numerous studies on CRISPR/Cas9 in embryos have highlighted its potential for treating monogenic diseases, alongside the risk of mosaic mutations and consequences that are on or off target. In a larger sense, CRISPR/Cas9 shows significant promise in treating monogenic diseases, which constitute over 80% of all rare diseases, often considered untreatable (Condò, 2022) . GGE through CRISPR could eliminate these diseases from individuals and future generations, benefiting around 6% of the population affected by monogenic diseases.

The CRISPR error rate, despite its potential, has led to the development of additional enzymes, such as SpCas9-HF1 and SaCas9-HF, to improve accuracy and decrease off-target effects. Zinc finger nucleases are used in other gene-editing techniques (Reardon, 2015). Common techniques for implementing CRISPR Cas9 include in vitro electroporation and microinjection. Applications in vivo encounter difficulties because of protecting decidua and incomplete knowledge of deleterious consequences. Mammals have been used to test novel in vivo GGE techniques such as transplacental gene delivery for acquiring genome-edited foetuses (TPGD-GEF) and enhanced genome editing by oviductal nucleic acid delivery (iGONAD). However, their application to human embryos is limited due to early understanding, monitoring challenges, and potential risks. Additionally, the presence of antiCas9 antibodies in donors poses complications for in vivo work with the system (Charlesworth et al, 2019).

Genome editing, while offering precise modifications, poses challenges for preventive medicine in clinical settings. Detecting small indels, including point mutations, resulting from off-target effects in the embryonic genome presents diagnostic uncertainties. Next-generation sequencing (NGS) like whole-genome or whole-exome sequencing, post whole-genome amplification (WGA), may be considered, but introduces artifacts. NGS struggles to distinguish small indels from single nucleotide polymorphisms or spontaneous mutations. Chromosome instability in embryos and potential off-target effects of nucleases in embryos with instability raise concerns about misleading preimplantation genetic diagnosis (PGD) results. Even if PGD confirms corrected genes in trophectoderm cells, the inner cell mass (ICM) may exhibit different genetic conditions. Risk assessment after germline genome editing is challenging. While clinical management post-embryo transfer might be considered, chromosomal mosaicism in the ICM could limit preventive measures. CRISPR/Cas9 treatment may exhibit low efficiency in correcting genes in polysomic cells, risking the beginning of hereditary illnesses in progeny.

Additionally, offspring subjected to embryo biopsy face a heightened risk of late-onset neurodevelopmental and metabolic diseases, as observed in recent mouse studies.

2.3: APPLICATIONS:

Within the realm of gene editing, CRISPER has several uses. It has been utilized to create homozygous animals with lack of function by mutating DNA. CRISPR is used to eliminate a specific domain from the Astl gene encoding ovastacin in order to determine the function of ovastacin in blocking sperm binding to zona pellucida after fertilization (Xiong et al. (2018). Generally speaking, mouse zygotes were treated with the CRISPR system (sgRNA and HDR oligonucleotide) in order to microinject the seven amino acids of the Ovastacin gene out. Mice with cataracts due to loss of the Crygc gene have been shown to be able to correct the deletion of the Crygc gene using the CRISPR system (Wu et al., 2013). Furthermore, CRISPR/Cas9-mediated genetic

repair of Duchenne muscular dystrophy (DMD) mutations was recently described. It has been confirmed that CRISPR in mouse zygotes can fix DMD mutations in a mouse model.(Min et al, 2019).

2.3.1: CRISPR System in Male Reproduction :

Animal model research in gene manipulation has been vital for evaluating reproductive processes, particularly in the context of gene disruption techniques like knock-out, which find successful application in the highly relevant biological system of reproduction. Specific genes crucial for fertility, often related to spermatogenic or haploid germ cells, are efficiently targeted using genome editing tools like CRISPR/Cas9. This technology has revolutionized genetic investigation, enabling the creation of knockout mouse models to explore male reproductive genetics and study essential components of the male reproductive system. The precision of CRISPR/Cas9 is highlighted, allowing for the simultaneous creation of multiple mutations, a capability previously unattainable through conventional methods. The application extends to the study of testis-specific genes, exemplified by the investigation of the Slx2 gene's role in spermatogenesis. Moreover, CRISPR/Cas9 exhibits promise in manipulating growing sperm cells for assisted reproduction, particularly in cases of male infertility due to maturation arrest. Notably, the system proves effective in repairing disease-causing mutations across species, showcasing its versatility in addressing genetic disorders like cataracts, Duchenne muscular dystrophy, and β-thalassemia. The successful application of CRISPR/Cas9 in rat spermatogonia gene modification further underlines its potential for targeted germline modifications, providing valuable insights into spermatogenesis (Khan et al., 2017).

2.3.2: CRISPR System in Female Reproduction :

CRISPR/Cas9 technology proves highly valuable in developmental studies of model organisms, demonstrating efficient genome editing in human embryonic cells. Recent applications include using CRISPR/Cas9 in tripronuclear zygotes to target and repair the β-globin gene, showcasing its potential in preventing genetic disorders. Despite the low efficiency of genome editing in embryos, studies in various animals, such as rats, sheep, cattle, pigs, and dogs, highlight the feasibility and effectiveness of this technology.

Human germline genome editing (HGGE) using CRISPR/Cas9 has been tested recently. Initially, gene editing was performed on human tripronuclear (3PN) zygotes, which are embryos with three pronuclei. Later, CRISPR genetic repair of endogenous β-globin gene (HBB) and glucose-6-phosphate dehydrogenase (G6PD) mutations was performed on these zygotes. With the development of microinjection technology, it is now possible to correct the MYBPC3 mutation in human metaphase II (MII) oocytes by co-injecting sperm and CRISPR/Cas9 components. The authors created an effective sgRNA and highly effectively targeted the POU5F1 (OCT4) gene using a zygote microinjection approach. They discovered that human blastocyst development failed as a result of OCT4 depletion, indicating that OCT4 in humans has regulatory functions during cleavage stages. We further emphasize that recent advancements have been made in the altering of human mtDNA. DddA-derived cytosine base editors (DdCBEs) are CRISPR-free editing techniques that have the ability to modify mtDNA in human cells. They catalyze C•G-to-T•A conversion by modified DddA deaminase, and they were first published in 2020. Next, Chen et al. edited the mtDNA in human 3PN embryos using the DdCBE approach. Particularly in oocytes and early embryos, mtDNA modification has drawn more attention and could be a busy area of study in the years to come (Zhang et al, 2023).

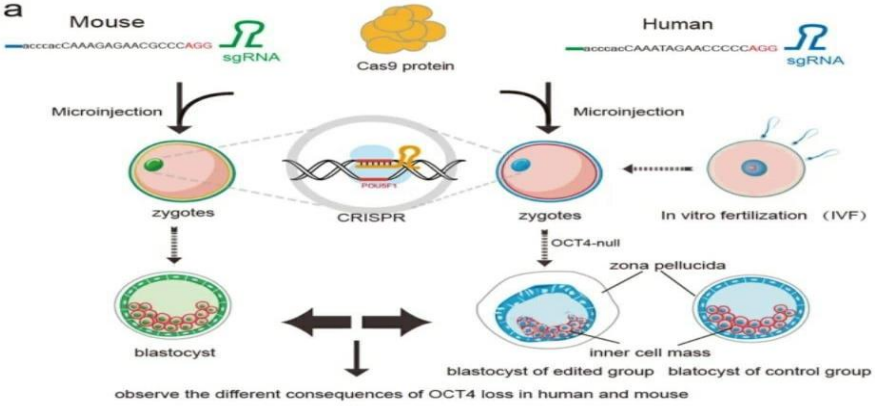

Fig 2: Diagram illustrating the experimental procedures for modifying the POU5F1 (OCT4) locus in human (blue) and mouse (green) zygotes by employing the CRISPR-Cas9 approach. The OCT4 gene's impacts on human embryo development were evaluated with and without it. Additionally, OCT4's function in mouse and human embryo development was compared (Khan, 2017).

Cas9 or TALENs microinjected into nonhuman monkeys produced transformed progeny, with zygote injection efficiency varying from 0.5% to 40.9%. In human zygotes, CRISPR was utilized, revealing both the specificity and fidelity of the system, although the edited embryos exhibited mosaic characteristics. Concerns about off-target mutations emphasize the need for further investigation, particularly when applying genome modifications during gametogenesis.The CRISPR/Cas9 system's potential extends to growing oocytes, offering a promising alternative for generating genetically modified oocytes in female germ line cells. This approach could address off-target mutations in subsequent generations. The technology has been successfully applied in pigs, introducing CRISPR/Cas9-mutated genes into somatic cells, demonstrating its versatility in modifying somatic cells and in vitro-produced zygotes (Khan et al, 2017).

Beyond genome editing, CRISPR/Cas9 creates new opportunities for gene drive system development, which might be used to control diseases carried by insects. This includes initiatives to disrupt Anopheles gambiae mosquitoes' capacity to spread disease, which could be a tactic in the fight against human malaria. Furthermore, by modifying the mitochondrial DNA of mice oocytes inserted into patient cells, CRISPR/Cas technology offers hope for the treatment of human mitochondrial illnesses. Although there may be advantages, there are also known concerns, such as the possibility that embryos won't be able to implant if the modified mitochondrial DNA is below a certain level (Khan et al., 2017).The precision, economy, and efficiency of today's genome editing technologies—especially CRISPR/Cas9—set them apart from earlier methods. The growing acceptance and application of assisted reproduction methods emphasizes their importance for modifying human embryos' genomes.(Khan et al., 2017).

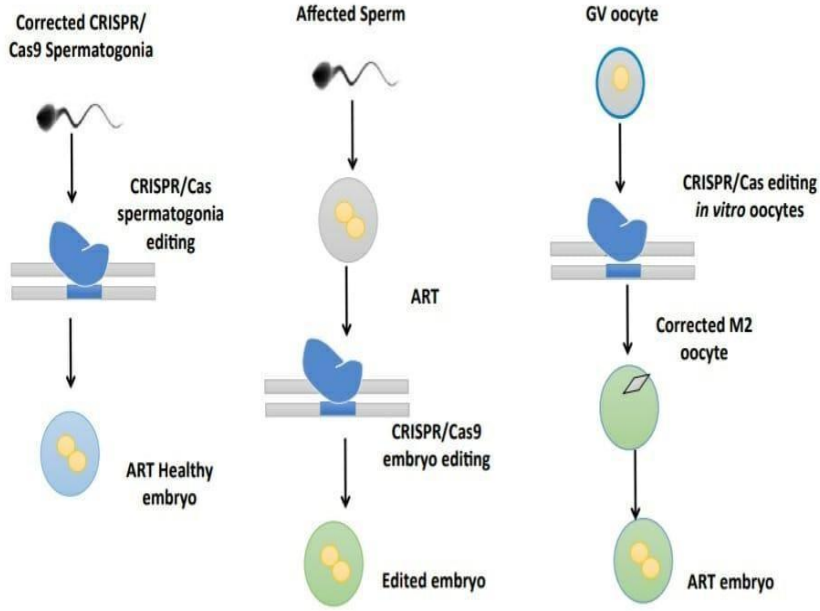

Fig 3: An illustration showing how to modify the genomes of sperm, oocytes, and embryos using CRISPR/Cas9. In order to create healthy embryos through artificial reproduction, the corrected sperm and oocytes can be employed. (Khan et al., 2017).

2.4: ETHICAL CONCERN:

Embryonic gene editing faces not only significant scientific barriers but also intricate social and ethical challenges. A primary ethical concern revolves around the alteration of the innate gene pool, raising uncertainties about the extended consequences of changes induced by Preimplantation Genetic Testing (PGT) in In Vitro Fertilization (IVF) and genome editing. International consensus on the ethicality of heritable gene editing remains elusive, with limited global discussion that primarily involves first-world western countries (Ledford, 2019).

There have only been a few experiments using CRISPR technology on human embryos, hence the field is still in its infancy (Vassena et al., 2016). Germline changes are viewed as unethical by many academics and members of the public, who emphasize that there is a "line that should not be crossed" (Cavaliere, 2016). There is concern that altered embryos could pass on their altered genome to subsequent generations, changing the genetic makeup of humans. Although it is morally imperative to take into account the consequences of current activities that may affect future generations, it seems too narrow-minded to limit

these precautionary considerations to modifications made to reproductive cells and embryos using genome editing technology. (Cavaliere, 2018).

From a patient's standpoint, the decision on whether to undergo genetic editing is deeply personal. Divergent perspectives exist, such as within the deaf community, where some view having deaf children as acceptable, resisting societal pressure to genetically edit deafness. Different people have different perspectives about what constitutes treatment and genome enhancement, so it's important to make that distinction.While their considerable support for editing the germline to treat incurable diseases, there is significant opposition to its use for enhancement purposes (Siddharth Manne,2023).

Concerns arise about multi-generational unforeseen effects on populations if CRISPR/Cas9 is employed for enhancing traits like eye color and muscle composition. Furthermore, ethical considerations extend to potential misuse of germline changes, especially with the removal of the

14-day limit on growing embryos in labs. The need for international consensus becomes paramount to balance the pursuit of understanding gene editing's embryonic implications while minimizing the risk of misuse in the future (Manne, 2023).

Despite the potential benefits of genome editing, it raises ethical concerns regarding the welfare of future children. Safety concerns and uncertainties about genome editing's safety compared to existing options, like PGD, divide scholars and the public.Whether or not to permit genome editing should involve a democratic process, considering differing views and values. The ethical assessment should not solely rely on a cost/benefit analysis but also evaluate existing alternatives, considering the future child's welfare in light of uncertainty (Cavaliere, 2018).

Procreative choices affect the kind and quantity of children produced, hence society interests play a critical part in determining whether genome editing should take the place of PGD, affecting a wider range of individuals. Despite historical suspicions and concerns related to eugenics, societal interests influence the governance of reproductive technologies.The governance of genome editing and other reproductive technologies must be democratic and deliberate, taking into account differing moral perspectives on these matters (Cavaliere, 2018).

2.5: LIMITATIONS.

The goal of ongoing CRISPR research is to boost productivity and look at new applications.

However, obstacles include high rates of mosaicism, low mutation repair effectiveness in embryos, and both on- and off-target impacts on the genome. The difficulties encountered must be carefully monitored and further studied for a comprehensive understanding of their impact on the safety of this technology (Reyes and Lanner., 2017).

When the DNA is cut by the Cas9 enzyme, off-target consequences happen at sites different from the intended target, potentially with similar sequences. While less error prone CRISPR mechanisms have been developed, concerns arise because much of the research in this area involves adult human cells or mouse embryos, which may differ greatly from embryonic human cells. The tolerable level of off-target effects remains a subject of debate, with varying opinions on the error rate for CRISPR on human embryos, as supported or challenged by different studies (Ledford, 2019).

Conversely, on-target effects of CRISPR occur at the intended site, relying on the cell's DNA repair pathways after cutting DNA. This repair can happen through non-homologous end joining, often causing DNA insertions or deletions, or through homology-directed repair (HDR), allowing researchers to rewrite a DNA sequence. Mosaicism arises as a significant issue when using CRISPR/Cas9 on human embryos, and accurately testing for it is challenging. Some older studies on human embryos indicate mosaicism in CRISPR-targeted embryos, complicating genome analysis and potentially leading to false positive results. Although clear strategies to eliminate mosaic mutations in CRISPR/Cas9-edited embryos are lacking, approaches like promoting Cas9 degradation and editing via ES cells show some advantages over zygote microinjection (Z. Tuel al, 2017).

Limitations of CRISPR/Cas9 were highlighted in a study by Alanis-Lobato et al. h, utilizing computational pipelines to analyse effects on preimplantation human embryos. Approximately 16% of the embryos analysed exhibited unintended gene editing outcomes in lengths of 4-20 kb. Loss of heterozygosity beyond target sites and segmental loss and gain of chromosome 6 were observed, emphasizing the need for careful scrutiny of CRISPR/Cas9 outcomes (Siddharth Manne,2023).

3. CONCLUSION.

The CRISPR system, with its basic elements, has become widely utilized in manipulating mammalian oocytes and embryos, facilitating scientific research and disease treatment exploration. This editing technology acts on mammalian genomes to correct mutations and enhance normal gene expression, yet faces challenges like off-target effects and on-target mutagenesis. Researchers seek to enhance efficiency by modifying the Cas9 enzyme, studying the nuclease working domain, and improving HDR rates. While CRISPR/Cas9 has been applied in animal models, ethical concerns and potential adverse effects limit its use in humans. Editing human embryos raises ethical dilemmas, and refining the technology for human genome editing may take years. CRISPR/Cas9's success in editing mtDNA in mice and humans offers insights into mitochondrial functions and related diseases. Addressing limitations, ethical considerations, and advancing detection technology will broaden and deepen its application in mammals, making it an increasingly valuable genome editing tool with minimized unexpected consequences.

The revolutionary CRISPR system is transforming the pace of genetic modification, correcting disease-causing mutations and generating disease-free individuals through the production of corrected mature sperms and oocytes. Its efficient application extends to creating synthetic gene drives for population-level control of vector-borne diseases. In the realm of genetic modification techniques, CRISPR/Cas9 stands out as the most efficient, accurate, and safe, particularly in human embryos, albeit requiring IVF and PGD for feasibility. IVF is undergoing rapid advancements through microfluidics and AI, enhancing gamete and embryo selection for a more efficient process. AI tools in IVF show promise in eliminating operator variability. Streamlining IVF can offer perceptions into the effects of CRISPR/Cas9 on in vitro embryos and facilitate scaled germline gene editing. PGT is crucial for understanding CRISPR/Cas9 applications in treating monogenic diseases, but it necessitates updated methods that differentiate between extraembryonic and embryonic cell lineages. Despite initial controversies and challenges like mosaicism and off-target edits, experiments using maternal DNA as an HDR template show promise for accuracy and efficiency. International consensus on genome editing in humans is essential to prevent misuse, with a clear distinction between treatment and enhancement. Research into applying CRISPR at early embryonic stages requires global collaboration to avoid controversy and ensure responsible use. While not yet consistently applied to human embryos, further research may unlock the potential of CRISPR/Cas9 in treating incurable monogenic diseases. Establishing a global consensus is imperative to guide future developments and promote ethical research practices.

Even though genome editing is a powerful genetic engineering technique, the genetics of the human germline and human genetic changes remain poorly understood, making its uncomplicated application in clinical settings now impractical. On the other hand, basic information about aneuploidy and mosaicisms can be obtained through genome editing, which will improve genetic counseling to assist infertile patients in making decisions about their reproductive options, aid in clinical management, and raise the success rate of assisted reproductive technologies.

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