Medical Ethics Approval Application Form

HarMoniCare Shenzhen Women's and Children's Hospital

<table>
<tr><td>Project</td><td colspan="3">CCR5 Gene editing</td><td>Duration</td><td colspan="2">March 2017 – March 2019</td></tr>
<tr><td>Classification</td><td colspan="6">Advanced technology / New Project
Class II or III medical technology
Research (√)
Reproductive Medicine
Organ transplantation
Others</td></tr>
<tr><td colspan="7">Applicant Information</td></tr>
<tr><td>Name</td><td>HE JIANKUI</td><td>Sex</td><td>Male</td><td>Education</td><td>Ph.D</td><td>Tel</td><td>18688955436</td></tr>
<tr><td>Research Directions</td><td colspan="6">Genomics</td></tr>
<tr><td>Application Description</td><td colspan="6">CCR5 is the gene which encodes a protein located on the surface of T cell wihch functions as a chemokine receptor for the immune system and plays a role in the binding of T cells to specific tissues and target organs. It regulates the migration, proliferation and immunity of T cells and monocytes or macrophages, and is mainly expressed on the membrane of resting silent T lymphocytes, monocytes, immature dendritic cells and so on. Results of population surveys and clinical studies indicate that individuals with CCR5 32bp-deletions have normal immune and inflammatory responses and are significantly resistant to multiple viral infections; therefore, gene editing on CCR5 may be effective in blocking cholera, smallpox or HIV infection.

Recently Chinese scientists made use of CRISPR-Cas9 to destroy hepatitis B virus. Gene therapy brings hope to rare but deliberating diseases. In February 2017, the US National Academy of Science, Engineering and Medicine released a statement that experimental study on the gene editing of embryos as therapeutics for the treatment of serious diseases is ethically acceptable. This brings hope to the treatment of many serious genetic diseases.

In this study, we plan to use the CRISPR-Cas9 to edit the embryo. Oocytes and sperms were collected from volunteers with HIV infection. CRISPR-Cas9 protein and gRNA are injected into fertilized eggs, and we can select the individual embryos with CCR5 gene edited by preimplantation genetic diagnosis, the edited embryos are transferred to women and pregnancy will follow. The baby born can gain the capability of resistance to HIV infection, smallpox, and cholera.

In early experiments, we have set strict quality control standards for gene editing</td></tr>
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	to fully assess the feasibility of CCR5 editing in embryo from a safety perspective. First of all, the cell lines and animal models (mice, monkeys) were used to conduct rigorous experimental studies on the effects of CCR5 gene editing. In particular, we chose monkeys, the species that is close to humans, as model animals for embryo editing by CRISPR-Cas9 method and assessed for the health status, physiology and neurobehavioral impact of the genetic editing. This design allows the identification of any related diseases due to gene editing. At the same time, we will isolate the embryonic stem cells post gene editing to detect for any abnormality of the proliferation and differentiation. Secondly, a variety of approaches are used to reduce off-target events and mosaic issues. For example, the high-fidelity CAS9 protein and the best sgRNA targeting CCR5 gene was used in combination with whole-genome amplification and genome-wide sequencing to detect off-target events and mosaicity. At the same time, we developed bioinformatics approaches to perform accurate assessment of the potential off-target harm. Finally, the multi-generational effects of gene editing are examined in animal models to explore the health status of the genetically-modified descendants Based on the above described research and experimental results, we designed this study aiming to perform in vitro fertilization using assisted reproductive technology, with the CCR5 gene being edited using CRISPR-Cas9 technology. The edited embryos are compared with the normal fertilized eggs to check for any difference in morphology. At the same time, the single-cell transcriptomics method will be used to compare the differences in transcriptome development between genetically modified embryos and normal embryos. Using PGS / PGD technology, we will perform rigorous genetic diagnosis and screening of the embryos before implantation by single cell whole genome sequencing to confirm that the editing is successful without off-targets and mosaic issues through thorough evaluation. We then choose the best embryo for implantation. During pregnancy, extensive physical examination is conducted and whole genome sequencing to be applied on the early- and mid-term amniotic fluid to make sure that the fetus is normal, until eventually a healthy baby will be born with CCR5 edited. Through this study, we expect to establish a solid technique standard for therapy by gene editing and bring gene editing related therapy to a new level. Ultimately, our research will stand out in the increasingly competitive international application of gene editing technology. This is going to be a great science and medicine achievement ever since the IVF technology which was awarded the Nobel Prize in 2010, and will also bring hope to numerous genetic disease patients.

Approval results	This study complies with the ethic regulations. We Agree to allow the conduct of the study. Signature by all Committee Members with the seal of the Chairman on 7th March 2017

INFORMED CONSENT

The research team is launching an AIDS vaccine development project. As the volunteer, your partner is diagnosed to have Acquired Immunodeficiency Syndrome (ADIS) or has been infected with Human Immunodeficiency Virus (HIV). Your health and other conditions are in line with the research's enrollment conditions. Therefore, the research team would like to invite you to participate in the research.

This Informed Consent will describe the goal, procedure, benefits, risks, inconveniences, rights and interests of the volunteers. Please read carefully and decide carefully whether to participate in the research or not. When the team explains and discusses the informed consent form to the volunteer, you can ask questions at any time and let him / her explain to you when you do not understand.

If you are currently participating in other clinical research, please be sure to inform the project team or researchers about the situation so as not to cause irreversible effects.

The project leader in charge of this project is **Jiankui He**, the source of the research funding is **Southern University of Science and Technology**.

Article 1 Why conducting this research?

1. The theoretical basis of the experiment: **Based on the human assisted reproductive technology, with the core of the CRISPR / Cas9 gene editing technology, gene editing of the CCR5 gene in the embryo would knock out the CCR5 gene. It would help these CCR5 gene editing babies to obtain the genotype of the Northern European to naturally immunize against HIV-1 virus;**

Method: Based on the human assisted reproductive technology, early embryos were injected with trace amounts of Cas9 RNP after intracytoplasmic sperm injection (ICSI) during normal IVF treatment. Cas9 RNP (Cas9 protein and optimized optical sgRNA) can act on the CCR5 gene, so as to prevent the newborn from the AIDS by editing the CCR5 gene and hindering the HIV-1 virus from invading the (CD4+) T cell. With the help of Preimplantation Genetic Screening or preimplantation genetic diagnosis (PGS / PGD), high-throughput whole genome sequencing, early pregnancy screening and other rigorous techniques could reduce the probability of birth defects, deformed children, etc., and decrease the risk of off-target issues and other risks. This technique may be able to produce IVF baby naturally immunized against AIDS (Referred to as baby) [See Annex I: Technical Procedure Flow Chart]

2. Technical Objectives: The main objective of this project is to produce infants who have the ability to immunize against HIV-1 virus.

Article 2 The research process, and the risk of adverse reactions.

1. Pre-test preparation

(1) You will undergo the following physical exams to determine if you can participate in the research 【See Annex II: Women Prior Check Items】. If the routine test of the medical situation is inconsistent with the basic contract of this research, the you should cooperate with the project team and the medical institution to conduct relevant tests. **Note: The rights and obligations in the testing shall be based on the contract between you and the medical institution.**

(2) Physician will check and record the volunteer's medical history. As volunteer, you have the obligation to truthfully disclose the relevant genetic history. The project team will not be responsible for any damage caused by the concealment of the related genetic medical history. [See Annex III: Female reproductive history]

2, Clinic trial process and risk (Note: The experiment process is a trade secret)

If the you pass the above process, **the project team will coordinate and recommend the medical institutions to conduct the following steps,** and describe in detail the risks or adverse reactions in each step. The details are subject to the contract signed by the volunteer and the medical institution.

	Technical route	Risk and adverse reactions	Note
① preparation-work	A. Preparation: both men and women are required to undergo physical examinations in the project designated medical institutions or the relevant department of obstetrics and gynecology clinic; more specific procedures shall be according to doctor's prescription; B. The project team has conducted ecomprehensive experiments using animal model and embryos previously to ensure the safety of the injected protein (Thermo Fisher) and sgRNA (SYNTHEGO) and the technique.	The project team conducts the pre-test inspections and operations, complying with the relevant provisions of the human reproductive center embryo laboratory to ensure that the processes of sterile are in line with embryonic laboratory procedures. No off-targets were found in our previous animal experiment.	Specific to you and medical institutions signed the contract shall prevail
②Controlled Ovarian Hyperstimulation (COH)	Fertility doctor will make individualized ovulation stimulation protocol according to patient's age, ovarian reserve function, hormone level, uterine tolerance and other factors. Patient would need to strictly follow the doctor's advice to inject the prescribed drugs and closely monitor follicular growth status by ultrasonic diagnosis. It is very important to inform the doctor if having any uncomfortable symptoms so that the	Adverse reactions include : A. Ovarian hyperstimulation syndrome: some patients may have symptoms of nausea, abdominal pain, ascites, pleural effusion, blood concentration or oliguria. Some severely reacting patients may have thrombosis, liver and kidney damage, or even life-threatening events. If this happens, drugs, surgery or other treatment may be applied to solve with	

	clinical doctor can adjust the treatment protocol immediately.	the emergency situation. B. Adverse reactions of ovarian: need to adjust the dosage of drugs, or even give up this cycle of treatment.	
③Eggs collection	A puncture needle will pass through the vagina to the edge of the ovary to collect follicles with B-ultrasonic guidance. Anesthesia is optional. After ovum pick up (OPU) , the follicles will immediately transferred to dishes with culture medium in embryo incubator for 5-6 days (37℃, 6% CO_2, 5% O_2 and saturated humidity).	Anesthesia risk, organ damage and retroperitoneal hemorrhage etc. may occur in follicles collection surgery process. Surgical interference should be taken if necessary. NO eggs could be collected through OPU because of patient's personal physical reasons would lead to an early end to the assisted reproduction cycle. Eggs collection or embryos transplant surgery carries a risk of infection, if this happened, anti-infective therapy would be used.	
④ In vitro fertilization and embryo culture	Intracytoplasmic sperm injection（ICSI）may be adopted strictly followed the guideline on human assisted reproductive technology by the National Health and Family Planning Commission of the People's Republic of China. The IVF procedure is supplemented with CCR5 gene therapy. Normal fertilized embryos will be cultured to blastocyst stage when 3-5 trophoblast cells will be biopsied from the embryo to perform whole genomic sequencing. The rest of the embryos will be frozen and conserved in liquid nitrogen.	Intracytoplasmic sperm injection (ICSI) would be applied for all the volunteers. Even though, the risks of fertilization failure may still exist. Embryos can't develop to the blastocyst stage because of sperm or egg quality problems, which may lead to no embryo, could be use in the next process. There is a possibility that some embryos may not have anti-AIDs ability. The doctor will give advice and the patient have the right to decide which embryos to be transplanted.	
⑤ Embryo Transfer	The trophoblast cell samples will be sent to testing centers to perform deep sequencing and bioinformatics analysis. Each embryo will be graded based on the totipotency and AIDS-resistant ability and full informed consent of the patients. 1-2 blastocysts will be transferred to uterus at appropriate time according to the woman's physiological cycle with the clinic doctor's advice. Progesterone should be used to prevent abortion.	See Article 3 for related risks.	

⑥Baby birth	2 weeks after embryos transfer, β-HCG hormone level will be checked. More than 50IU/ml means successful biochemical pregnancy. Pregnant women need to monitor gestational sac, fetal heartbeat, and the pregnant women can be sent to the obstetric care center. In case of three or more fetuses, the fetus reduction treatment may be needed according to the guidelines of the State Family Planning Commission and the sconsideration of erious harm of multiple births to mothers and infants. At the same time do early chorionic aspiration and post-birth related screening. Fetal cells or cell-free DNA depth sequencing, maternal vaginal loss cell test, amniocentesis and other tests could be implemented to assess each embryonic development potential and the ability to fight against AIDS.	See Article 3 for related risks.	

Article 3 Possible risks and precautions.

1. With the existing knowledge, the additional operations proposed in this project will not put the mother at risk of contracting HIV and other infectious diseases. In case of any disputes over rights and obligations during the project period between you and the medical institution, the contract signed shall prevail, and the project team is not responsible for this.

2. In order to ensure normal embryo development, in vitro fertilization and incubation phase injection of trace substances is at the trace amount (pg level, that is 10^{-10}–10^{-12}g). It does not guarantee that gene editing will play its role. It is normal if the infants do not have the capacity of natural Immunity to AIDS. The project team does not assume legal responsibility in this situation.

3. The primary risk of gene editing (DNA-targeted CRISPR-Cas9 endonuclease) is the off-target effect of generating extra DNA mutations at sites other than the intended target. This is due to that the technique can cause nonspecific cleavage, resulting in mutations in non-targeted genomic sites. **PGD, whole genome-wide sequencing, amniocentesis and peripheral blood test of mothers in different stages of pregnancy after transplantation will minimize the possibility of substantial injury.** Therefore, this project team is not responsible for the risk of off-target which is beyond the risk consequences of the existing medical science and technology.

4. Since the male volunteer in this project are infectious patients, even though the use of IVF as a means of pregnancy has the least possibility of transmission of the virus to the mother or

the baby, it cannot completely remove the risk of infection. The risk is **Not** caused by this project, and this project team will not take responsibility.

5. **Pregnant women volunteers are required to go to the designated medical institution (tentatively designated as Shenzhen Luohu Medical Institution) at the 9th month of pregnancy to prepare for delivery.** The project team is responsible for paying the rent, and for work-related expenses for the pregnant women and one of their nursing staff (at 200 RMB/ day). After the baby is born and up to 28 days, they need to live in the production ward of the designated medical institution. The expenses incurred by the medical institution during the period are borne by the project team. The project team also pays the worker's mistress and one of the nursing workers' (at 200 yuan / day), until the completion of a variety of examinations for newborns. The project team will also purchase health insurance for the baby; **if in the process of pregnancy the volenteers incur with other diseases or complications unrelated to the test, they are responsible for the cost of the corresponding treatment.**

6. Neonatal malformations, congenitally deficiency, suffering from common genetic diseases belong to the scope of natural risk of natural reproduction, the project team does not assume legal responsibility.

7. Regarding the qualitative characterization of the project results, only the project team has the right of final interpretation and announcement to the public. Then you have **NO** right to explain and have **NO** right to announce the project or result information without permission. Violation of this will dealt as breach of contract and the volunteers need compensate for the damages (The specifics are defined in the liquidated damages cooperation agreement).

8. Maternal infection with the HIV virus may cause fetal infection. The project team conducts routine testing and deep gene sequencing, **but cannot completely rule out the risk of HIV based on maternal infection in infants.** This is a common problem of childbirth in patients with infectious diseases and does not belong to the risk caused by the project. The project team is not responsible for it.

9. In case of force majeure, laws and regulations, policies and reasons of the university, the project team needs to suspend or terminate the trial. The volunteers who have already incurred the expenses in the previous period need not to give back to the project team and are not liable for additional compensation.

Article 4 The impact of participating in the study on volunteers' daily lives.

1. When you decide whether or not to participate in the research, please carefully consider the possible impact of the above examinations and follow-up on your daily work and family life. Consider the time of each visit and traffic problems. You can consult with us if they have any questions about the tests and steps involved in the clinical trial.
2. Research requires that female volunteers actively fulfill their obligations as follows:
   (1) Before taking any new prescription drugs, consult your physician and members of the

project team; In case of physical discomfort during pregnancy, promptly inform them and do not make the decision by yourself.

(2) Taking into account your safety and the validity of the test results, you can no longer participate in any other clinical studies on drugs or medical devices during the study period.

(3) Taking into account the safety and the validity of the test results, you need to get regular pregnancy tests at the Tier A municipal-level hospitals in accordance with Annex IV [See Attachment IV: Birth Control Project]. The pregnancy test record files are scanned and mailed to the project team, and mailing costs reimbursed by the project team.

(4) In the research process, husband and wife can not conduct unprotected sexual intercourse (primarily referring to the pregnancy period).

(5) One month prior to the expected due date, you need to go to the designated medical institution (Luohu Medical Institution, Shenzhen) and stay there waiting. You will be required to stay there for another 28 days after the delivery.

Article 5 Possible benefits of participating in the research

1. This research project will likely help you produce HIV-resistant infants. It is beyond the scope of the research project to test the HIV-related effects of maternal infection during the research period, and the risk is borne by volunteers. HIV resistance in infants is based on a health certificate issued by a post-natal medical institution obstetric.
2. The project team purchases Ping An Group's Anxingbao insurance for babies born.

Article 6 Whether it is necessary to participate in and complete this research?

1. Whether you participate in this research is entirely voluntary. If you do not want to, you can refuse to participate, and this will have no negative impact on your current or future health care. You may withdraw from this study at any time without any pre-requisite prior to completing the COH. However, after entering the first test-tube cycle, you will naturally withdraw from this project under certain circumstances as follows: (2), (3) and (4). When you decided not to participate in this study, the research team would hope that you would inform the research doctors in time and the research doctors may provide advice and guidance on the volunteer's health. Once there is any information that may affect the volunteer's decision to continue participating in the study, the project team will inform you in time.
2. After COH and before embryos implantation, if you decide to withdraw from this project, then the costs incurred in the previous period will not need to be reimbursed back to the project. But the contract is terminated and the latter part of the cost will not borne by the project team.

3. After the first IVF cycle with embryo implanted, no pregnancy occurred or spontaneously aborted after pregnancy, they could restart the second cycle. If still no pregnancy or abortion occurs after the second cycle, then the contract terminates. The project team will pay 2,000 RMB nutrition fee to the volunteer. The other contract you have with the medical institu-

tions shall prevail.

4. The husband and wife should communicate with the doctors when the fetus was found with genetic defects or other serious diseases after the embryos implantation in the first IVF cycle. They could start the second cycle after the abortion if clinic doctor recommends. If the couples face the same problems in the second IVF cycle, the project team will pay the abortion costs and nutrition fee of 2,000 RMB to terminate the research study. The other contract with the medical institution shall prevail.

5. After the embryo implantation in the first cycle of IVF until 28 days post-birth of the baby, if you decide to leave the study due to other reasons than the ones listed in Items 3 and 4 above, you will need to pay back all the costs that the project team has paid for you. If the payment is not received within 10 calendar days from the issuance of the notification of violation by the project team, another 100,000 RMB of fine will be charged.

Article 7 Who is responsible for the cost of participating in the research?

1. The costs incurred in this clinical trial are paid by He Jiankui laboratory at the South University of Science and Technology under the project fund. The coverage details are as follows:
   (1) IVF outpatient costs, COH costs, embryo laboratory culture costs;
   (2) Round-trip transportation costs (limited to high-speed rail second-class seat or economy class airfare);
   (3) Accommodation costs: 350 RMB / day / person;
   (4) Lost work allowance: 200 RMB / person;
   (5) Pregnancy detection and monitor costs: Villus puncture, amniotic fluid test, B-ultrasound etc. (For details, please refer to Attachment 4: Project Examination. I understand and agree with my legal wife to do the above tests.)
   (6) The cost of awaiting medical treatment at the designated medical institution one month prior to the birth and the post-natal recovery at the designated medical institution within 28 days after birth, as well as the rent and nursing fees (200RMB / day);
   (7) Insurance: The project team will buy Ping An Group's A-Star health insurance for the newborn baby.

2. Based on the calculation of the average costs of such a procedure, the project team commits to pay total cost of 280,000 RMB per couple. Any cost beyond this limit will be the responsibility of the volunteers. Any costs incurred during the trial but unrelated to the research, including but not limited to the treatment of other diseases and travel accident will be the responsibility of the volunteers.

Article 8 Research-related injuries

When the volunteer's health is impaired due to participation in this research, please inform the project team members (Chen Yuanlin Tel: 15013147861 or Song Shuo Tel: 18565856308). We will immediately take the necessary medical measures to protect the health of the volunteers. Ac-

cording to the provisions of the relevant laws and regulations of our country, in the event of injury related to the research, the project team will bear the corresponding medical expenses and provide corresponding economic compensation with the limit of 50,000 RMB.

Chapter9 Confidentiality of volunteers' personal information

1. The volunteers' personal data in the research are kept confidential. The number ID instead of the volunteer's name will identify samples collected from the volunteer's body. Information that identifies you will not be disclosed to anybody other than the project team, unless approved by volunteers. When necessary, government departments may check the volunteer's personal information in the project. At the time of publication of this research, your identification information will not be disclosed.

2. If you quit from the research, the project team will keep your information until the final destruction. The information will not be used or disclosed. However, in the rare occasions below, the project team will continue to use or disclose your information even if you have left the research or the research has finished. These situations include: a) the removal of your information will affect the scientific soundness of the research results or the evaluation of data safety; b) limited information for research, teaching or other use (the identification information will not be included, such as the volunteer's name, identity card Numbers, or other personal information that identifies a volunteer); and c) requested by the university and government regulators for overseeing the research.

Article 10 Rights of the project team

1. After birth, the project team or the medical institution should preserve the umbilical cord blood for later use. After the baby is born, it needs to cooperate with the project team or the medical institution to conduct a series of routine tests. For details, see Appendix 5: Post-natal Maternal and Infant Test Project

2. Baby's photo on the day of birth will be kept by the project team. The project team has the portrait right of the infant and can make it open to the public. [See Attachment VI: Portrait License for Use]

3. The baby's blood samples need to be disclosed to the public. If parents are willing to disclose their portrait and name, their wishes prevail.

4. Regarding the project results, only the project team has the right of final explanation and announcement to the public. The volunteers have no right to explain, publish, or announce project related information without permission.

Article 11 Confidentiality clause.

1. For the project team's trade secrets, you hereby agree:

(1) You strictly observe the secrets of the project team and take all the security measures and systems to protect the secret;
(2) You cannot disclose any commercial secrets to any third party;
(3) You shall not use the secret at any time except for the purpose of fulfilling the contract with the project team;
(4) You can not copy or use the secret through reverse engineering.

Both the project team and volunteer, please read the relevant statement carefully and sign it.

Project team statement

"I have informed the volunteer about the research background, purpose, steps, risks and benefits of the AIDS Development Research Project, giving him/her enough time to read the informed consent, discuss with others, and answer questions about this research; I have told the volunteer to contact the (project leader) whenever they have problems related to the research, and to contact them at any time when they have problems related to their rights/equity, and provide accurate contact information; I have informed the volunteer that he/she can withdraw from the study without any reason."

Signature of the project team leader Date

Volunteer statement

"I have been informed of the background, purpose, steps, risks and benefits of the research on the HIV Development Research Project. I have enough time and opportunity to ask questions, and I am very satisfied with the answers to the questions. I was also told about the person to contact if I have questions, want to report problems, give suggestions and provide help for research, or want to get further information. I have read this informed consent and agreed to participate in this study. I know I can I withdraw from the study at any time during the study without any reason. **I am fully aware of the above mentioned study and all signatures have legal effect."**

(Please write the last sentence after reading)

———————————————————————————————————————

Signature of Volunteer Receiving informed consent
Date

Citizen identification number:
Address:

Annex I: Technical process

IVF is an advanced assisted reproductive technology, and the specific process is as follows:

1. Controlled ovarian stimulation COH	Because the length of natural menstrual cycle varies from person to person, and there are also differences in different cycles of the same patient, it is not easy to arrange the time of fetching eggs, and there is only one dominant follicle in the natural cycle. After fertilization, only one embryo can be formed, while the pregnancy rate of implanting a single embryo is very low. Therefore, it is necessary to use controlled superovulation to enhance and improve ovarian function so as to achieve the goal of obtaining multiple healthy eggs without the limitation of natural cycle, provide multiple embryos for implantation, and synchronize luteal development with endometrial function as far as possible. Controlled superovulation usually uses GnRHa to decrease FSH and LH in the body, then HMG or FSH to stimulate follicular growth in the ovary. The dosage of drugs is adjusted according to the patient's reactivity to drugs. The age and dosage of drugs are different, so will be the number of eggs obtained.
2. Monitoring follicles	In order to evaluate the stimulating effect of ovary and determine the time of fetching eggs, the size of follicles should be monitored by vaginal B-mode ultrasonography, and E2 value (estrogen) should be checked by blood sampling to adjust the dosage of drugs. When the diameter of two or more follicles is greater than 1.8 cm and the number of follicles over 1.4 cm is equal to E2 value, human chorionic gonadotropin (hCG) can be injected to promote follicle maturation. The eggs were taken at 34~36 hours after injection of hCG.
3. Egg retrieval	The most commonly used method of egg retrieval is under local anesthesia (optional without anesthesia, but also a common surgical method in China). Under the guidance of vaginal B-mode ultrasound, the fetching needle goes through the vaginal fornix and reaches the ovary directly to absorb the eggs. Then embryologist will immediately transfer these eggs under a microscope to a petri dish containing the embryo culture medium, and culture these in an incubator at 37 °C.
4. Semen collection	The time taken for sperm removal is the same as that of egg collection. Wash your hands before taking semen by masturbation. The cup is sterile. Do not touch the rim or inside of the cup, mark the donors husband and wife's name clearly, take sperm time, and check the identity information with the laboratory embryologist (routine IVF baby steps). The sperm was treated by upstream method or by density gradient centrifugation to obtain sperm with good motility.
5. ICSI and gene editing	Matured eggs were fertilized with single sperm intra-cytoplasmic sperm injection (ICSI) 4 to 5 hours after the collection of the eggs. Co-injection of Cas9 protein (Thermo Fisher) and CCR5 sgRNA (SYNTHEGO) with the sperm suspension.
6.embryo culture	Normal fertilized zygotes are cultured in an embryo incubator with the suitable pH culture medium, temperature, humidity and CO2 concentration. The embryo development is recorded according to the principle of human assisted reproductive technology human embryo processing, and the embryo development potential is evaluated.
7. Preimplan-	After 5-6 days embryo culture, 3-6 embryonic trophoblast cells are biopsied for PGD at blastocyst stage, and a small number of samples are taken for whole genome se-

tation genetic diagnosis and whole genome sequencing	quencing to detect possible genetic diseases, gene editing efficiency and off-target problems to ensure healthy embryos could be transferred.
8. Embryo transfer	The vagina and cervix are cleaned with saline and then rinsed with culture medium. A metal or plastic catheter is placed in the cervix. A 1.3 mm Teflon tube is used to aspirate embryo cells from the culture medium under a microscope and 0.03-0.05 ml of culture medium or 0.03 -0.05 ml of serum should be supplied with together. The tube was inserted into the cervical canal and placed the embryo into the uterine cavity at the base of 0.5cm. After embryo transfer, the woman need to stay in bed for 2-4 hours, restrict activities for 3-4 days, and receive progesterone treatment. The β-GCG assay was performed 2 weeks after transplantation, and if there was no pregnancy, the progesterone was stopped. The successful pregnancy case is a key target for monitoring.
9. Clinical evaluation	After the transplantation, it can be judged whether the pregnancy is a success in 2 weeks.

Above is the general process of performing IVF. As for the detailed plan, the doctor will formulate according to the actual situation of the patient.

Annex 2: Women's examination list prior to the research (please provide detailed version)
The inspection items that the woman should do:

Item 1	Blood test: blood type, blood routine test, clotting time, erythrocyte sedimentation rate, sex hormones (FSH, LH, E2, P, T, PRL), thyroid function, preoperative four items (hepatitis B, hepatitis C, AIDS, syphilis), TORCH, anti-sperm antibody (AsAb), anti-endometrial antibody (EmAb), anti-phospholipid antibody (ACA), anti-ovarian antibody, liver and kidney function, fasting blood glucose, chromosome, thalassemia screening, tumor markers (CA125 , CEA, AFP), stool routine + occult blood.

Item 2	Vaginal/cervix secretion examination: leucorrhea routine, mycoplasma, chlamydia, gonococcal, cervical cancer prevention test (cervical smear or TCT), HPV virus detection.
Item 3	Fallopian tube examination (if there is already indication of IVF, such as male obstructive azoospermia, extremely few, weak, abnormality, etc., it is not necessary to perform fallopian tube examination), chest X-ray, electrocardiogram, vaginal B-ultrasound, abdominal B-ultrasound (Hepatobiliary, spleen, pancreas and kidney). In some cases, the blocked antibody is examined at the doctor's advice.
Item 4	The woman's initial examination time can be on the 2-4th or the 10th day of menstruation.
Item 5	Long-term effective examination: chromosome, blood type, fallopian tube angiography
Item 6	Effective examination within half a year: four items preoperative (hepatitis B, AIDS, syphilis, hepatitis C), mycoplasma, chlamydia, gonococcal, abdominal B-ultrasound (hepatobiliary, spleen and pancreas)
Item 7	Follow-ups are generally valid for one year

Above is a general examination item for in vitro fertilization surgery. As for the detailed plan, the doctor will formulate according to the actual situation of the patient.

Annex III: Female assisted reproductive medical records

Female Assisted Reproductive Medical Record Home Page

Name of woman, age, occupation, education level, national identity card (or passport) number:
Husband's name, age, occupation, education level, national identity card (or passport) number:
Mailing address: Postal code:
Contact cell phone number:
Chief complaint:

Current medical history
Past medical history: Hepatitis: ☐ No ☐ Yes, Tuberculosis: ☐ No ☐ Yes, Kidney disease: ☐ No ☐ Yes, Cardiovascular disease: ☐ no urinary tract, urinary tract infection: ☐ no ☐, history of sexually transmitted diseases: ☐ no ☐, Appendicitis: ☐ no ☐ yes. pelvic inflammatory disease: ☐ no ☐ yes, History of surgery: ☐ No ☐ Yes, others:
Personal history: smoking: ☐ no ☐ yes, #sticks / day, alcoholism: ☐ no ☐ yes, drug abuse: ☐ no ☐ yes, Accustomed medication: ☐ No ☐ Yes, history of drug allergy: ☐ No ☐ Yes, History of major mental disturbance: ☐No ☐ Yes, Health status: birth defects in the past: ☐ no ☐ Yes,
Menstrual history: menarche age __, menstrual cycle__ days, menstrual volume: ☐ normal ☐ more ☐ less dysmenorrhea: ☐ yes ☐ no
Marriage and Childbirth History: Close relatives marriage: ☐ Yes ☐ No; Remarriage: ☐ No ☐ Yes; Pregnancy ☐ No ☐ Yes, the last pregnancy time and year; Pregnancy, artificial abortion, spontaneous abortion, drug flow, induction, premature birth, Ectopic pregnancy: left, right Full-term maternity, existing child, adopting children: ☐No ☐Yes。
Family history: history of genetic disease ☐ no; ☐ have (detailed) History of infertility ☐ no; ☐ have (detailed)

Female assisted reproductive medical records (2)

Physical examination	Vital Sign： T ℃, P times/min, R times/min, BP KPa
	Height cm, weight kg, body mass index
	Nutrition: ☐ normal ☐ abnormal_____
	Development: ☐ normal ☐ abnormal_____
	Mental: ☐ normal ☐ abnormal_____
	Hair: ☐ normal ☐ abnormal_____

	Skin and mucous membranes: ☐ normal ☐ abnormal_____
	Lymph nodes: ☐ normal ☐ abnormal_____
	Breast: ☐ normal ☐ abnormal Galactorrhea: ☐ yes ☐ no
	Heart: ☐ normal ☐ abnormal_____
	Lung: ☐ normal ☐ abnormal_____
	Liver: ☐ normal ☐ abnormal_____
	Spleen: ☐ normal ☐ abnormal_____
	Kidney: ☐ normal ☐ abnormal_____
	Spinal limbs: ☐ normal ☐ abnormal_____
	Others:
Gyneco-logical exami-nation	Vulva: ☐ normal ☐ abnormal
	Vagina: ☐ normal ☐ abnormal
	Cervical: ☐ smooth ☐ smashed (☐ light ☐ medium ☐ heavy) Nasal cyst (☐ no ☐ have) hypertrophy (☐ yes ☐ no)
	Uterus: ☐ front position ☐ rear position ☐ flat size: ☐ normal ☐ abnormal Texture: ☐ Soft ☐ Medium ☐ Hard activity: ☐ Activity ☐ Restricted Fixed tenderness: ☐ Yes ☐ No
	Uterine attachment: left side: ☐ normal ☐ abnormal right side: ☐ normal ☐ abnormal Trichomonas: ☐ Yes ☐ No mold: ☐ Yes ☐ No Cleanliness: Grade I, Grade II, Grade III
	Others

Female Assisted Reproductive Medical Record (3)

Pre-IVF routine examination				
Blood test	Hemoglobin	g/L	Red blood cell count	$\times 10^{12}$/L
	White blood cell count	$\times 10^{9}$/L	Blood cell volume	%
	Platelet	$\times 10^{9}$/L	ESR	mm/H
Urine examination				
Blood group			Rh	
Coagulation	PT		KPTT	

Sex hormones	FSH miu/ml	E_2 pg/ml	P ng/ml
	PRL ng/ml	LH miu/ml	T ng/ml
TORCH	Toxoplasma gondii	Cytomegalovirus	Rubella virus
	Herpes simplex virus		
Hepatitis B	HBsAg	HBsAb	HBeAg
	HBeAb	HBcAb	HBcAb-IgM
HCV	HCVAb		
HIV	HIVAb：		
Syphilis			
Serum antisperm antibody			
Liver	GPT U/L	GOT U/L	
Kidney	Serum cretonne	Blood urea nitrogen	
Chromosome			
Cervical scraper			
Cervical smear	Chlamydia		
Endometrial biopsy			
Hysteroscopy			
Laparoscopic			
Uterine fallopian tube fluid			
Hysterosal-pingography	Uterine morphology		
	Left fallopian tube right fallopian tube		
Electrocardiogram			
Gynecological B-ultrasound			

Female Assisted Reproductive Medical Record (4)

Natural cycle ovulation monitoring before treatment:

Date
Menstrual cycle
Follicular diameter
Endometrium

Diagnosis:

Medical treatment plan:

Physician:

Date:

Human assisted reproductive technology

Common inspection items after entering the cycle

Item	Time	Note
Ovulation induction drug injection	Cycle begins	Different schemes have different durations and charging standards (1 week to 2 weeks,

		10,000-20,000 RMB)
B-ultrasound detection of follicular development	COH to OPU	
OPU	DAY0, OPU day	
Follicle retrieve	DAY0, OPU day	In general, 15 eggs are obtained to meet the needs of blastocysts culture.
Egg freeze	DAY0, 2-4 hours after OPU	No sperm available or meet frozen egg indicator
Sperm wash	DAY0, 2-4 hours after OPU	
Sperm freeze	DAY0, OPU day or other day	The patient can choose to freeze sperms for the cases of difficult-to-collect semen, microsurgery sperm collection, or azoospermia.
IVF insemination	DAY0, 3 hours after OPU	
ICSI	DAY0, 6 hours after OPU	For those who can not use IVF insemination or have fertilization obstacle issues
Granulocyte demolition	DAY0, 4-6 hours after IVF insemination or for ICSI	Monitor the release of the second polar body. Less than 1/3 of the total number of mature eggs requires R-ICSI.
R-ICSI	DAY0, failure of IVF insemination	After fertilization, when the second polar body is less than 1/3 of the total number of mature eggs requires R-ICSI.
Pronuclear check	DAY1 16-20 hours after Insemination	
Embryo culture	DAY1, Replace culture medium after pronuclear observation	Record all embryos condition, droplet culture
Cleavage stage check	DAY3, Cleavage stage check	Observe the embryos, communicate with the clinician to make decision for blastocysts culture, fresh embryos freezing, or fresh embryo transfer
Blastocyst culture	DAY3-DAY7 Replace culture medium	droplet culture
Embryo transfer	DAY3 DAY4 DAY5-DAY7	DAY3-DAY4 cleavage transfer DAY5-DAY7 blastocyst transfer

Embryo freeze	DAY3 DAY5-DAY7	DAY3, cleavage embryo freeze DAY5-DAY7 blastocyst freeze
Embryo thaw	DAY3 DAY5-DAY7	DAY3 cleavage embryo thaw DAY5-DAY7 blastocyst thaw
Progesterone injection	Transfer day	prevent miscarriage
Serum hormone assay	2 weeks after transfer day	β HCG >50IU/ml biochemical pregnancy
B-ultrasound monitoring	4 weeks after transfer day	Pregnancy sac and fetal heart monitoring

Annex IV: Pregnancy examination list

First check	Before 13 weeks	Established "Pregnant Women's Health Handbook", urine HCG, gynecological examination, blood routine, urine routine, blood sugar, thyroid function, electrocardiogram, B-ultrasound (NT screening), (high-risk pregnant women eligible for peripheral blood NIPT test) (fasting)
Follow-up	12-16 weeks	Amniocentesis: It is necessary to do the most accurate means of screening for genetic diseases in designated medical institutions, and to screen out off-target problems that may be caused by genetic editing. This is the most accurate method used as quality control standard.
Follow-up	16-18 weeks	Obstetric examination (includes fetal heart rate Doppler), blood type (ABO, Rh), blood routine, urine routine, renal function (3 items), liver function (5 items), hepatitis B two and a half, hepatitis C virus antibody, syphilis serum Antibody, blood homocysteine, hemoglobin electrophoresis experiment (Ground Pot Screening, G6PD), high-risk population nutrition analysis (fasting)
Follow-up	20-24 weeks	Obstetric examination, urine routine, color B-ultrasound (two-dimensional or three-dimensional)
Follow-up	24-28 weeks	Obstetric examination, urine routine, blood glucose screening, (high-risk population) premature birth prediction (fasting), (Note: blood glucose screening requires 10 hours of fasting at night, blood is taken on an empty stomach at 8:00 in the morning, followed by drinking glucose water for 5 minutes. Take blood samples at 1 hour and 2 hours after the start of drinking.).
Follow-up	28-30 weeks	Obstetric examination, urine routine, ABO antibody test (if the pregnant women's type is O, their husbands should be tested for ABO antibodies if their types are A, B, or AB)
Follow-up	30-32 weeks	Obstetric examination, urine routine, blood routine, color Doppler ultrasound. The pregnant women should monitor the fetal movements starting from Week 30. Fetal movement is an important sign of the fetus' safety. Every night between 6-10 o'clock, the woman should count for 1 hour, and the number of fetal movements per hour should be 3-5 times. If the number of fetal movements per hour is less than 3, or the fetal movement is reduced by half compared to previous, and the fetal movement is sudden and frequent, it should continue for another hour. If the problem remains, the woman should go to the medical institution.
Follow-up	32-34 weeks	Obstetric examination, urine routine, and some high-risk groups re-check for 75g OGTT.
Follow-up	34-36 weeks	Obstetric examination, urine routine, fetal heart monitoring (detection of fetal heart hypoxia by fetal monitor on fetal heart when the fetus moves)
Follow-up	37 weeks	Obstetric examination, urine routine, B-ultrasound, blood routine, liver and kidney function, fetal heart monitoring, (electrocardiogram) (fasting)

Follow-up	38 weeks	Obstetric examination, urine routine, fetal heart monitoring
Follow-up	39 weeks	Obstetric examination, urine routine, fetal heart monitoring)
Follow-up	40 weeks	Obstetric examination, urine routine, fetal heart monitoring)

Annex V: Routine testing items after birth of a baby

(1) Newborn registration (newborn name, father's name, mother's name, gender, date of birth, home address, BCG vaccination time, telephone, etc.);
(2) Weight, body length, appearance, crying, skin jaundice and birth record;
(3) Identification of genotypes by toes blood at 7 days after birth.

Annex VI. Licensure of the Use of the Portrait

Licensor Name, Gender, Birth ID by the Delivery Hospital
Guardian of the Licensor: _____

Licensee (Institution): The Project Team by Dr. He Jiankui
Address:

I agree that: Professor He Jiankui and his project team organize the photographing of me (Guardian:). The copyright of all photographs and image materials (hereinafter referred to as portrait materials) which contain his portrait belongs to Professor He Jiankui and his project team. The authorized use period is within ten years from the date of the birth of the fetus, and the photographs and images of the portrait materials belong to Professor He Jiankui and his project team. The original is kept by Professor He Jiankui and his project team (authorized person). All or part of the profits obtained by the authorized person from using the portrait data or the portrait data contained in the portrait data belong to the authorized person, and I do not require participation in the distribution.

I can not retrieve the authorization: Professor He Jiankui or his designee has the right to use in all kinds of media, both at home and abroad for profit or non-profit purposes (including: 1, newspapers, magazines, books, calendars, pictures, charts, etc.; 2, Internet, LAN, application software; 3, TV, wall, car body inside and outside, elevators, outdoor billboards, various audio-visual broadcasting equipment; 4, propaganda; Manual, product packaging; 5. All other legitimate media or media can use all or part of their own portraits contained in the portrait materials (including for advertising, trademarks, logos, decorative windows, article matching, etc.).

Professor He Jiankui and his designated project team do not need to notify me when they use all or part of the portraits in accordance with this authorization.

This authorization is non-revocable. It has two copies and each party holds one.

Signature of Guardian of Licensor

Signature of Licensee

Date:

Location:

Supplementary explanation of informed consent

(Long-term health follow-up plan)

This note is a supplementary explanation of informed consent. If there is any inconsistency with the original document, this statement shall prevail. Any unsettled matters shall be discussed separately by the two parties.

As a novel technology, gene therapy has brought about revolutionary effects for some diseases which are difficult to cure. However, there are still some uncertain factors for gene therapy, including theoretical off-target effect, efficacy and persistence of treatment. Based on the premise of respecting individual autonomy, our research group formulated an 18-year health follow-up plan to ensure the normal development and healthy growth of volunteers' children in a conscientious and responsible manner. The examination tests listed in this plan can be increased or reduced according to the actual situation.

The cost of the following examinations is borne by our research group. If there are no special instructions, they will be tested in the nearest first-class hospitals. If abnormalities caused by gene editing occur in the test, further treatment should be carried out in the corresponding first-class hospitals. All the treatment costs will be borne by insurance companies and our research group (no amount limit). Within nine months before and after the birth of the child, participants should consult the doctor immediately if any accident occurs. Even if it is not related to this study, the project team will also bear the corresponding compensation, but it is limited to 50,000 yuan.

Each examination report is given in two copies. The volunteer and our research group each hold one. In order to maintain a balance between ensuring maximum transparency and respecting patient privacy, the team will keep the identity information of volunteers and their children in strict confidentiality.

First physical examination

Time: day of birth

[Routine examination items]

1. Body weight, body length, appearance, crying, and jaundice on the skin.
2. Heart rate
3. Reaction after stimulation
4. Muscle tension
5. Breathing

[Special examination items] (Note: Jiankui He's laboratory is responsible for these items).

1. Umbilical cord blood was collected to check the editing efficacy of CCR5 gene editing.
2. Umbilical cord blood was sent to perform a virus infection test to confirm the antiviral effect.
3. Whole genome sequencing of DNA extracted from umbilical cord blood for screening genetic diseases (including thalassemia, albinism, phenylketonuria, hemophilia, etc.).
4. The umbilical cord and placenta samples were collected to detect chimeras.

Second physical examination

Time: 28 days after birth

[Routine examination items]

1. Height and weight
2. The development of all parts of the body.
3. Evaluating developmental intelligence.

Third physical examination

Time: 6 months after birth

Physical examination items: weighing, measuring height, measuring head circumference, measuring chest circumference, visual acuity, audiometry, examination of motor development, oral examination, evaluation of developmental

intelligence, blood test (routine blood test), bone examination, trace element examination, detection of HIV infection.

Fourth physical examination

Time: 1 years after birth

Physical examination items: weight, height, head circumference, chest circumference, anus, sitting alone, crawling, pronunciation, movement development, oral examination, the number of teeth, evaluation of intelligence development, bone examination, HIV infection detection. Babies are prone to get anemia and ascaris lumbricoides infection. During the physical examination, hemoglobin and stool should be checked.

Fifth physical examination

Time: three years old

Physical examination items: weighing, measuring height, measuring head circumference, visual acuity, oral examination, blood routine examination, HIV infection detection.

[Special inspection items] (Note: Jiankui He's laboratory is responsible for these items).

The blood was sent to perform a virus infection test to confirm the antiviral effect.

Sixth physical examination

Time: five years old

Physical examination items: weighing, measuring height, measuring head circumference, visual acuity, oral examination, blood routine examination, bilirubin combination, liver function test, internal medicine, surgery, ophthalmology examination, HIV infection detection.

[Special examination] bone age determination

Seventh physical examination

Time: seven years old

Physical examination items: weighing, measuring height, measuring head circumference, visual acuity, oral examination, blood routine examination, bilirubin combination, liver function test, internal medicine, surgery, ophthalmology examination, HIV infection detection.

[Special inspection items] examination of West Nile virus and hand foot and mouth disease

Eighth physical examination

Time: 10 years old

Physical examination items: weighing, measuring height, measuring head circumference, visual acuity, oral examination, blood routine examination, bilirubin combination, liver function test, internal medicine, surgery, ophthalmology examination, HIV infection detection.

[Special examination] IQ measurement

Ninth physical examination

Time: 13 years old

Physical examination items: height, weight, blood pressure, color discrimination, visual acuity, sensory organs (ear, nose and throat), internal medicine, surgery, chest X-ray, blood routine, urine routine, liver function 11 items, kidney function 3 items, blood lipid 2 items, fasting blood sugar, trace elements 7 items. If necessary, check ECG, B-mode ultrasound, chest X-ray, gonorrhea, AIDS, ophthalmic examination + slit lamp, fundus, ear, nose, throat, oral cavity, etc.

Tenth physical examination

Time: 17.5 years old

Physical examination items: height, weight, blood pressure, color discrimination, visual acuity, sensory organs (ear, nose and throat), internal medicine, surgery, chest X-ray, blood routine, urine routine, liver function 11 items, kidney function 3 items,

blood lipid 2 items, fasting blood sugar, trace elements 7 items. If necessary, check ECG, B-mode ultrasound, chest X-ray, gonorrhea, AIDS, ophthalmic examination + slit lamp, fundus, ear, nose, throat, oral cavity, etc.

When the volunteer's children reach the age of 18, the informed consent of the health follow-up plan should be signed by the volunteer's children themselves.

Signature of the volunteer: The head of the research group signed:

Date: Date: