Note S2

Animated informational videos for improving public understanding of research involving human embryos or stem cells

We created an original animated video to explain research activities involving human stem cells or embryos for the public attitude survey presented in the main text. Our video explains the following issues: stem cells; embryo models; research involving the transplantation of human iPSCs into pig embryos to create pigs with organs derived from human iPSCs; research involving aborted fetal cells or tissues; in vitro fertilization; research involving the fertilization of germ cells generated from iPSCs; mitochondrial replacement in human embryos for pregnancy; and human embryo culture beyond 14 days. We considered how this video improved the public understanding through the following procedure:

A web-based survey for the general public living in Japan was conducted including 16 correct/incorrect questions that tested the respondents' understanding of the research activities involving human stem cells or embryos, and responses to the items involved selecting one of the following three options: "Yes," "No," or "I don't know." The inclusion criteria for participation in this survey were age 20–69 years and no response to the attitude survey were presented in the main text.

The respondents were assigned to three groups. Group A completed the survey after watching the original video, whereas Group B completed the survey after receiving illustration- and text-based explanations. Group C completed the survey without receiving any explanation. The target number of participants was 400 in each of the three groups (a total of 1,200).

The explanations in Group B largely comprised transcriptions of the video content and one or two illustrations that were also present in the video for each issue. Groups A and B were asked about their level of subjective understanding of the explanation provided. Participants responded using a four-point scale with the following options: "Easy to understand" (level 4), "Relatively easy to understand" (level 3), "Relatively difficult to understand" (level 2), and "Difficult to understand" (level 1).

We commissioned the Nippon Research Centre for data collection. Participants were recruited from a sample of people who voluntarily registered with a panel at the Nippon Research Center. Data were collected from February 9 to 17, 2022, until the number of participants reached 1,200. We aimed for the age and sex distributions of the sample to be consistent with those of the general Japanese population in each group. Therefore, the target numbers were set in 10-year increments according to sex. First, data from the respondents assigned to Group A were collected. Once the target sample size was reached by age and sex, data for Group B were collected, followed by those for Group C. For example, for males in their 20s, the first 32 individuals who accessed the survey screen were assigned to Group A, the next 32 to Group B.

When tabulating the data, we organized the responses to the 16 questions into correct and incorrect answers, with "I don't know" treated as an incorrect answer. We analyzed the median and 25–75th percentiles of the total number of correct answers in each group. The Kruskal–Wallis test was employed to assess the statistical significance of the differences in the total number of correct answers among the three groups. Subsequent analyses using Dunn's test verified specific significant combinations. To account for multiple comparisons, the Bonferroni correction was employed to adjust the p-values.

Furthermore, since the level of subjective understanding (but not their objective understanding) is often asked by participants in attitude surveys, we performed Spearman's rank correlation coefficient test between their levels of subjective understanding (4-point scale) of the explanation and the total number of correct answers. Significance was set at a p value of 0.05 (5%). Data were analyzed using IBM SPSS Statistics for Windows version 27 (IBM).

Table S1 presents the median and quartiles of the total number of correct responses for each group as well as the results of the comparison between the groups. The median and 25–75th percentile values were the highest in Group A (11 and 9–13), followed by Group B (10 and 6.25–12). Comparisons between the groups indicated significant differences for all combinations ($p < 0.01$ in all combinations).

Table S1. Medians of the total number of correct answers and comparison between groups

	Median	25–75th percentile	Comparison between groups†
Group A: Video	11	9–13	Vs. B ($p < 0.01$) Vs. C ($p < 0.01$)
Group B: Illustration and text	10	6.25–12	Vs. A ($p < 0.01$) Vs. C ($p < 0.01$)
Group C: No explanation	4	1–7	Vs. A ($p < 0.01$) Vs. B ($p < 0.01$)

† Kruskal-Wallis test was performed to confirm significant differences, and Dunn's test was used for comparisons between groups. P-values were adjusted using Bonferroni correction.

Table S2 shows the relationship between the level of subjective understanding of the explanations and the total number of correct answers in Groups A and B. Spearman's rank correlation coefficient was 0.41 for Group A and 0.43 for Group B ($p < 0.01$ in both groups). A significant correlation was observed in both groups. Thus, the higher the subjective understanding, the higher the actual number of correct answers for both groups.

Table S2. Relationships between subjective understanding and the total number of correct answers in Groups A and B

	Subjective understanding level						
	Level 4	Level 3	Level 2	Level 1	rs	95% CI	p
Group A: Video	129 (32.3%)	169 (42.3%)	73 (18.3%)	29 (7.2%)	0.41	0.32–0.49	< 0.01
Group B: Illustration and text	58 (14.5%)	183 (45.8%)	106 (26.5%)	53 (13.3%)	0.43	0.35–0.51	< 0.01

rs, Spearman's rank correlation coefficient; CI, confidence interval.

This study has several limitations. For example, we only used one or two illustrations in each part of the explanation provided to Group B. It is possible that the number of correct answers in Group B would have been higher if more illustrations were used. However, videos have been suggested to improve public understanding.

Questionary for the survey of the Note S2(translated in English)

[SC1] Please select your gender.

1.Female

2.Male

[SC2] Please select your age.

1.0～19

2.20～29

3.30～39

4.40～49

5.50～59

6.60～69

7.70～

［SC3］ **Did you respond to the Survey of Public Attitudes About Stem Cell or Embryo-Related Research, which was conducted with a response period of January 5, 2022 to January 14, 2022?**

1. Yes

2. No

Request for responses to our web-based survey

This survey is conducted by the Japan Research Center, Inc. and was commissioned by the University of Yamanashi.

In recent years, the status of medical research involving human subjects has been changing rapidly, with particularly remarkable progress in regenerative medicine and stem cell research.

Regenerative medicine refers to medical treatments aimed at artificially manipulating the regenerative ability of tissues to restore impaired tissues and organs to their normal state. Experiments are being conducted on the use of human cells and fertilized ovum (embryo) for realizing regenerative medicine treatments. However, because of various bioethical concerns, there are ongoing efforts to promote international alignment on what types of experiments are acceptable. In addition to the opinion of researchers, that of patients, their families, and the general public is also important. This requires the dissemination of easy-to-understand information.

The purpose of this survey is to examine what means of explanation would help the public better understand stem cell or fertilized ovum (embryo) research. Those who cooperate in the survey will be divided into three groups: (1) a group that will watch a video explanation, (2) a group that will read an illustration- and text-based explanation, and (3) a group that will not be given any explanation. The correct answers to the questions will be provided in the final screen at the end of the survey. You are assigned to (1) a group that will watch video explanations / (2) a group that will read illustration- and text-based explanations/ (3) a group with no explanations. We apologize for the inconvenience this may cause you, but we appreciate your cooperation.

1. The purpose of the web-based survey

We will investigate what means of explanation would help improve the public's understanding of stem cell and fertilized ovum (embryo) research.

2. Target audience for the web-based survey

Registered survey panel members.

3. Use of results, protection of personal information

Your answers will be compiled into a statistical number, such as "XXX is the percentage of respondents who answered 'XXX'." Your name and personal information will not be disclosed. Your personal information will not be handled by the University of Yamanashi, which is the survey administrator. The results will never be used for any purpose other than research purposes.

4. The total number of questions

About 20 questions.

＊For the video group

There are some questions in the survey that you will be asked to answer after watching the video and based on contents from the video.

Please make sure that upon answering the survey you are in an environment in which you can play the videos and audio before answering the questions.

Please read the above text carefully and select whether you are willing to participate in this survey or not.

1. Yes
2. No

〇iPSCs.

[Q1-1] Stem cells in the body can transform into a variety of cells, just like iPSCs.

1.Yes

2.No

3.I don't know

[Q1-2] iPSCs are created by manipulating fertilized ovum (embryos).

1.Yes

2.No

3.I don't know

〇The embryo model.

[Q2-1] Embryo models are created by fertilizing a sperm and an ovum, just like a real fertilized ovum.

1.Yes

2.No

3.I don't know

[Q2-2] Embryo models function similarly to real fertilized ovum and can be used to gain knowledge that improves infertility treatment among other areas.

1.Yes

2.No

3.I don't know

〇Research involving the transplant of human induced pluripotent stem cells (iPSCs) into pig embryos to create pigs with organs derived from human iPSCs.

[Q3-1] Human iPSCs are implanted into a fertilized ovum of a pig that has been genetically modified not to produce a pancreas; the fertilized ovum is implanted into the pig's uterus. A piglet born in this way does not have a pancreas because it is genetically modified to not produce a pancreas.

1.Yes

2.No

3.I don't know

[Q3-2] Human iPSCs are implanted into a fertilized ovum of a pig that has been genetically modified not to produce a pancreas; the fertilized ovum is implanted into the pig's uterus. A piglet born in this way has a pancreas derived from human iPSCs.

1.Yes

2.No

3.I don't know

◯Research involving aborted fetal cells or tissue.

[Q4-1] Tissue obtained from aborted fetuses can be used in various fields, including regenerative medicine research.

1.Yes

2.No

3.I don't know

[Q4-2] With the creation of iPSCs, tissue from aborted fetuses is no longer necessary for research. ・

1.Yes

2.No

3.I don't know

◯In vitro fertilization.

[Q5-1] The fertilization of sperm and ovum in a culture dish is called in vitro fertilization.

1.Yes

2.No

3.I don't know

[Q5-2] Fertilized ova created by in vitro fertilization can be used for fertility treatment as well as for experiments in culture dishes.

1.Yes

2.No

3.I don't know

◯Research involving the fertilization of germ cells generated from iPSCs.

[Q6-1] Sperm and ova can be created from iPSCs.

1.Yes

2.No

3.I don’t know

[Q6-2] Embryo models are created by fertilizing sperm and ova generated from iPSCs.

1.Yes

2.No

3.I don’t know

〇The use of mitochondrial replacement human embryos for pregnancy

[Q7-1] A child’s mitochondria are inherited from their mother.

1.Yes

2.No

3.I don’t know

[Q7-2] In order to prevent diseases caused by mitochondrial mutations from being passed on to the child, the mitochondria of the mother’s ovum are removed and replaced with mitochondria from the donor’s ovum.

1.Yes

2.No

3.I don’t know

〇Research involving the culturing of human embryos in vitro beyond 14 days.

[Q8-1] The primitive streak, which appears in the fertilized ovum about 14 days after fertilization, is the source of the nerve.

1.Yes

2.No

3.I don’t know

[Q8-2] The use of fertilized ova 14 days after fertilization for research is prohibited in many countries. However, in recent years, a prominent international society has recommended that such laws be revised.

1.Yes
2.No
3.I don’t know

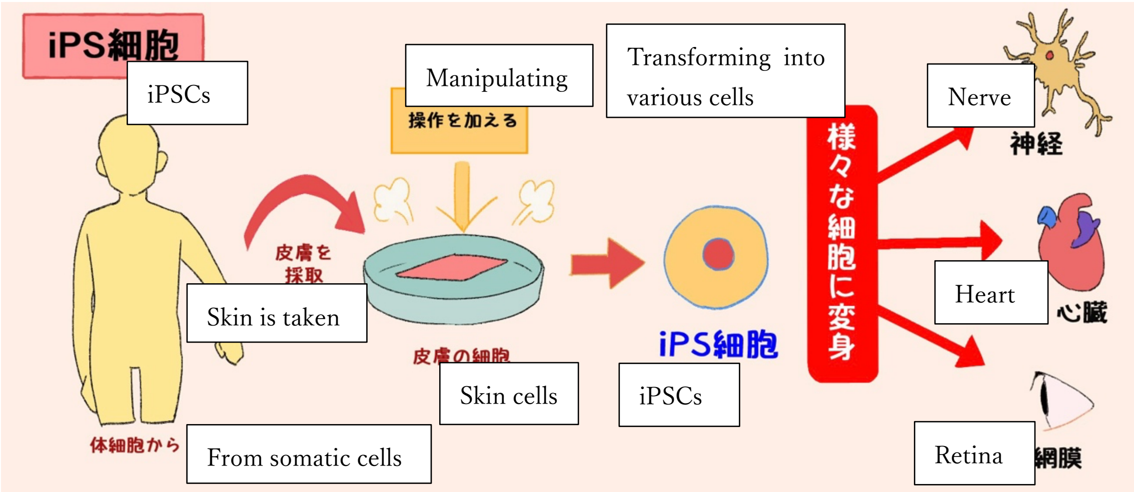
（*For video explanation group and illustration- and text- explanation group）

Q. Do you think the explanation was easy to understand?

1. Easy to understand
2. Relatively easy to understand
3. Relatively difficult to understand
4. Difficult to understand

Illustration- and text-based explanations (translated in English)

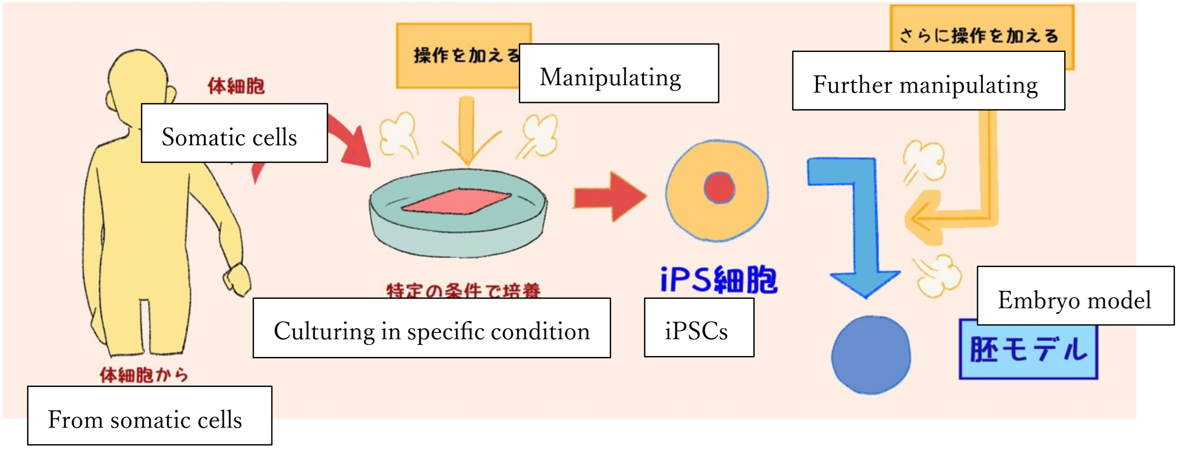
Part 1: Definition of stem cells and prerequisite knowledge regarding the research activities involving stem cells, some of which are described in Parts 2–4.



Our bodies started as a fertilized ovum -- in other words, an embryo: a single cell. As this single cell divides and transforms, organs such as the heart are formed. Although heart and other organ cells continue to divide, they remain heart cells no matter how much they divide. In contrast, stem cells can change into other types of cells. We distinguish between stem cells that are present in the body and stem cells that are made in a culture dish. There are several types of stem cells in the human body. Each has a role in maintaining the organ where they are located, but they only create cells that form that particular organ. If we use the blood as an example, hematopoietic stem cells create red blood cells, platelets, cells that control immunity, and all the other cells that make up the blood. The most well-known type of stem cell that is created in a culture dish is iPSCs. Today, I will restrict my description to iPSCs.

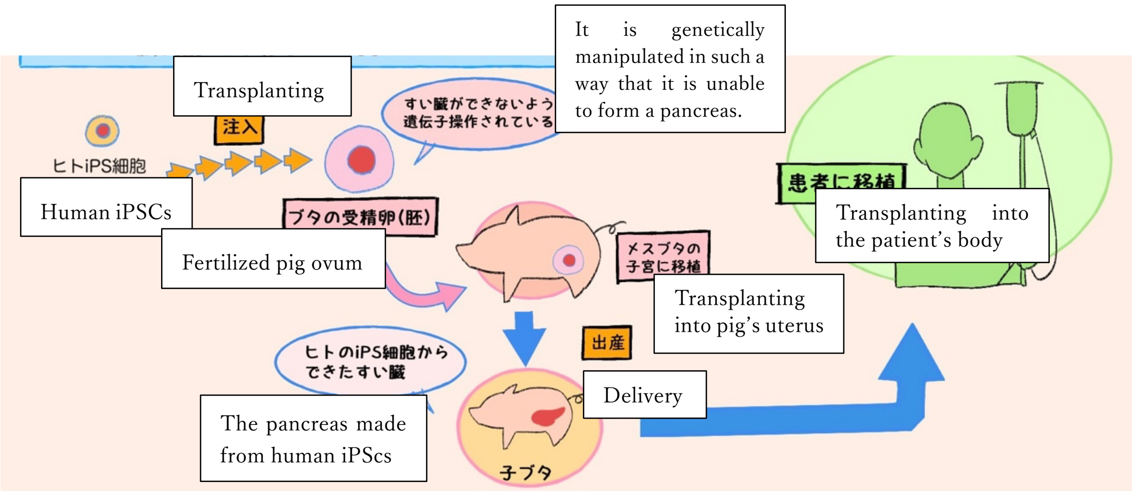
iPSCs are made by manipulating somatic cells in the body such as skin or other cells, and they can then be transformed into a wide variety of cells -- such as nerve, heart, and retina cells -- through further manipulation. This is a major difference from stem cells in the body. Stem cells such as iPSCs are used as materials in regenerative medicine. For example, efforts are being made to create a treatment method in which retina cells are made from iPSCs and then transferred into eyes suffering from an illness.

〇**Part 2: The embryo model.**



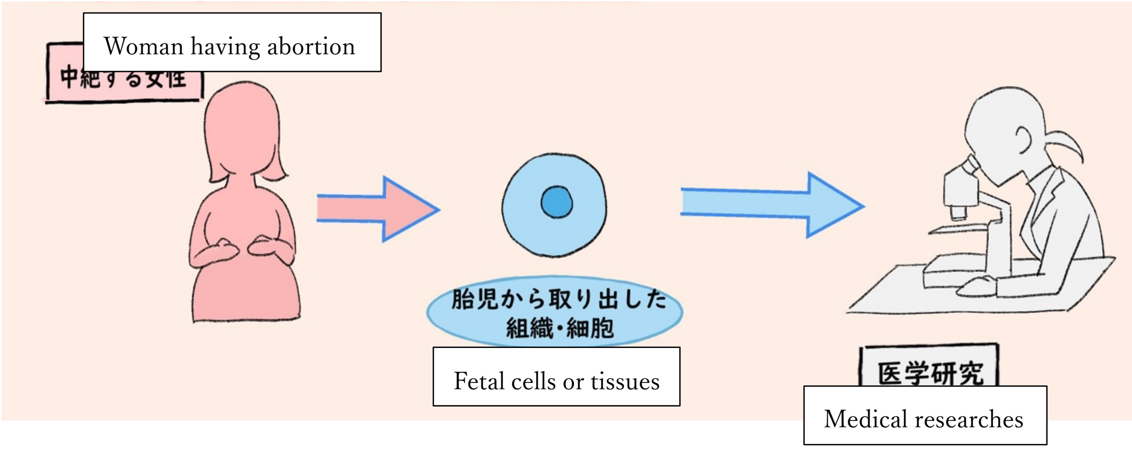
We can create embryo models by manipulating iPSCs. An embryo model is not an actual fertilized ovum, but it functions like a fertilized ovum. Of course, embryo models are not transferred into women's uteruses as they are only created for use in culture dishes during experiments. Experiments using embryo models do not involve real fertilized ova, but they can provide us with knowledge that will improve infertility treatments.

Part 3: Research involving the transplant of human induced pluripotent stem cells (iPSCs) into pig embryos to create pigs with organs derived from human iPSCs.



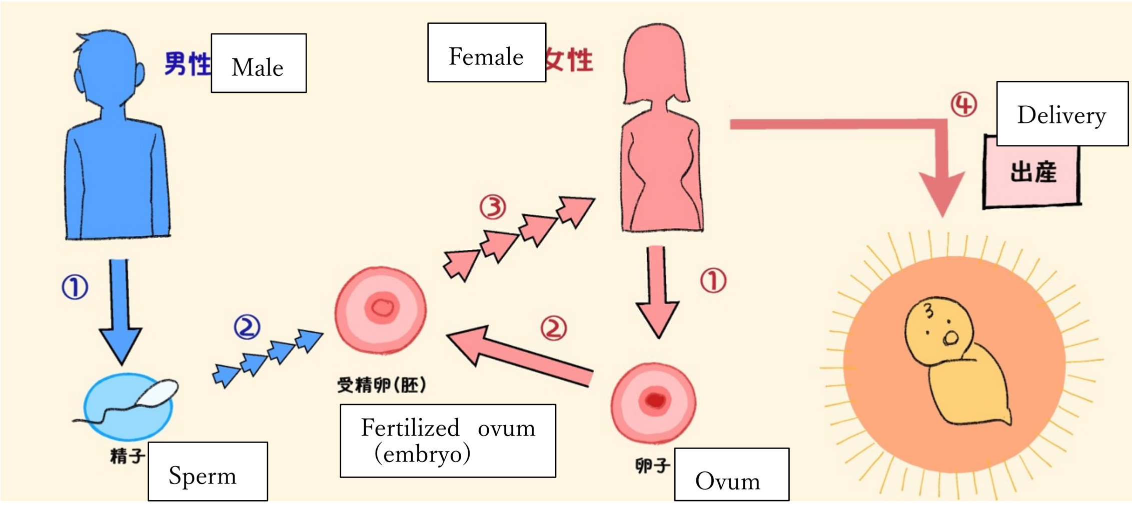
It is conceivable that human organs could be created in the bodies of animals. For example, there is a fertilized pig ovum that is genetically manipulated in such a way that it is unable to form a pancreas. Even if such an ovum was transferred into a sow's uterus, the piglet that is eventually born would not have a pancreas. However, human iPSCs could be introduced into the ova. Then, it is transferred into a pig's uterus. Once this is done, the piglet that is eventually born would have produced its pancreas from human iPSCs. Although organs created in this way can be used in research to identify the causes of diseases, the final objective is to transfer them into individuals with illnesses. Even though it is possible to create pancreas cells from iPSCs, it is difficult to assemble these cells into a pancreas. That is why the above mentioned method has been considered.

Part 4: Research involving aborted fetal cells or tissue.



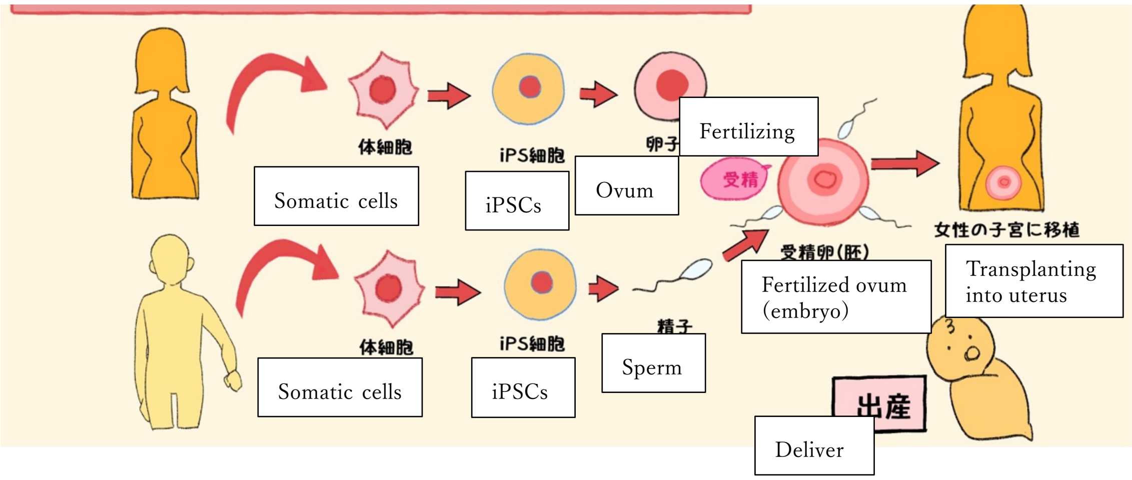
It is a different issue than iPSCs. Stem cells and other tissues and cells can be obtained from aborted fetuses. In cases in which the objective cannot be fully achieved through the use of cells such as iPSCs, these tissues and cells can be used in experiments. Specifically, they are used in research such as regenerative medicine as well as treatments and vaccines for infectious diseases.

Part 5: Explanation of in vitro fertilization, which is prerequisite knowledge for research activities involving human embryos, some of which are detailed in Parts 6–8.



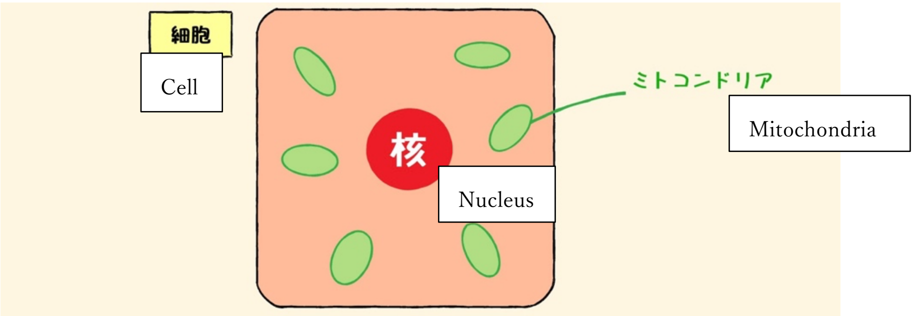
First, we obtain sperm and an ovum. Then, we fertilize the ovum. The result is a fertilized ovum; that is, an embryo. Once this fertilized ovum is transferred into a woman's uterus, she'll become pregnant and give birth. This method is known as in vitro fertilization-embryo transfer. It is widely used as an infertility treatment. Sometimes, the fertilized ovum is not transferred into a woman's uterus but is instead used for experiments conducted in a culture dish. Once the experiment is finished, the fertilized ovum is discarded.

Part 6: Research involving the fertilization of germ cells generated from iPSCs.

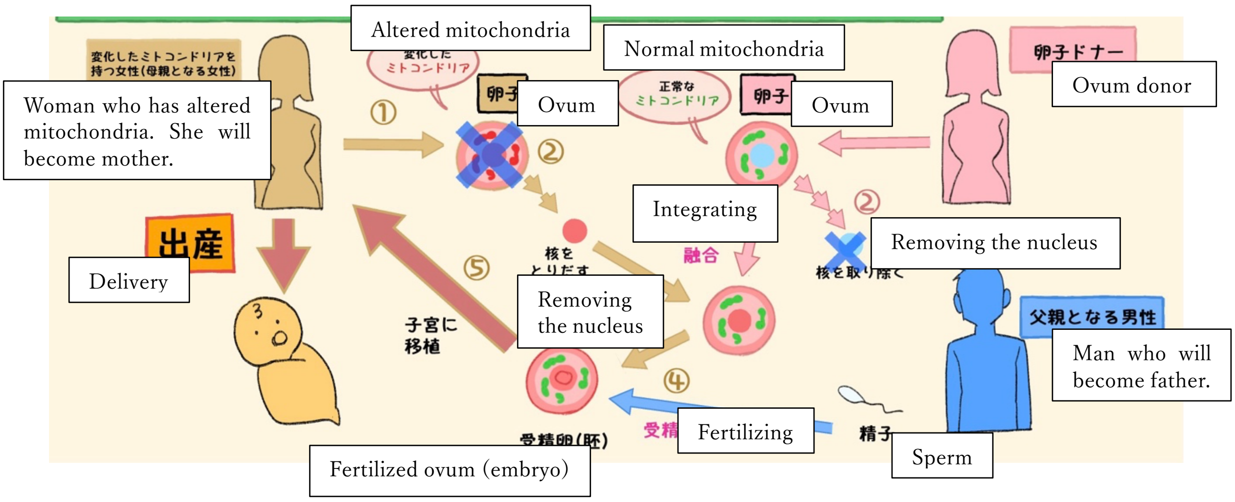


Ovum and sperm can be created from iPSCs. We may create fertilized ovum using such sperm or ovum. If we use fertilized ovum made in this manner in experiments, we can obtain knowledge that can be used in the development of infertility treatments. In addition, if the fertilized ovum is then transferred into a woman's uterus, she may become pregnant and give birth, so this technology may be used as a type of infertility treatment.

Part 7: The use of mitochondrial replacement human embryos for pregnancy.

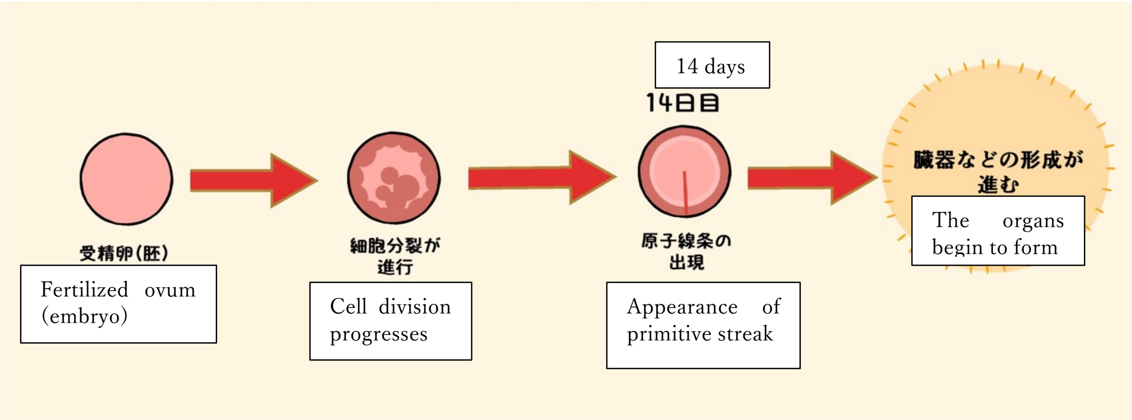


Mitochondria are included in each of the cells of our body. They are inherited by children from their mothers. If mitochondria undergo alteration, they can sometimes cause illness. This next example is of a method used to prevent a child from inheriting altered mitochondria from his or her mother.



First, an ovum from the woman with the altered mitochondria -- that is, the woman who will become the mother -- is obtained. Then, we remove the nucleus from this ovum. Next, a ovum is obtained from a donor woman who has normal mitochondria. The nucleus from this ovum is removed and integrated with the nucleus taken from the ovum of the woman who will become the mother. The parts of the ovum other than the nucleus from the woman who will become the mother and the nucleus from the donor woman's ovum will not be used any longer. Then, sperm from the man who will become the father is used to fertilize the ovum outside the body (in vitro) to make a fertilized ovum. This fertilized ovum is then transferred into the uterus of the woman who will become the mother. The mitochondria of the child who is later born will be inherited from the woman who served as the donor.

Part 8: Research involving the culturing of human embryos in vitro beyond 14 days.



When a fertilized ovum is used in an experiment, one of the problems that we face is how long we can use this ovum after fertilization. The rule currently utilized in countries around the world is that such an ovum can be used up to 14 days. Around 14 days, a structure known as the primitive streak -- which will eventually become the nerves throughout the body -- appears. Then, the organs will begin to form. The formation of the primitive streak is defined as the moment that the formation of a unique human being. However, recently a prominent international association has expressed the view that the use of a fertilized ovum beyond 14 days may be allowed. Advances in technology made it possible to culture fertilized ovum beyond 14 days, and to study the details of the process by which a fertilized ovum becomes a fetus.