【Grant-in-Aid for Specially Promoted Research】

# Elucidation of the mechanism for human oocyte development based on its *in vitro* reconstitution


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## Purpose and Background of the Research

### ●Outline of the Research

The germ-cell lineage creates new individuals, perpetuating the genetic information across the generations. We have been investigating the mechanism for germ-cell development, and have shown that mouse ESCs (mESCs)/iPSCs (miPSCs) are induced into primordial germ cell-like cells (mPGCLCs) with a robust capacity both for spermatogenesis and oogenesis and for contributing to offspring. These works have served as a basis to unravel key mechanisms of germ-cell development such as epigenetic reprogramming/programming, sex determination, and meiotic entry.

By investigating cynomolgus monkey development, we have defined a developmental coordinate of pluripotency among mice, monkeys, and humans, identified the origin of the primate germ-cell lineage in the amnion, and have elucidated the X-chromosome dosage compensation program in primates. Accordingly, we have succeeded in inducing human iPSCs (hiPSCs) into human PGCLCs (hPGCLCs) and then into oogonia with appropriate epigenetic reprogramming. We have also shown that hPGCLCs can be propagated to ~$10^6$-fold over a period of 4 months under a defined condition.

Building on these achievements, this research aims to achieve *in vitro* reconstitution of human oocyte development (induction of oocytes at ovarian follicle stages) and establish a foundation for understanding the mechanism of critical events associated with human oocyte development.

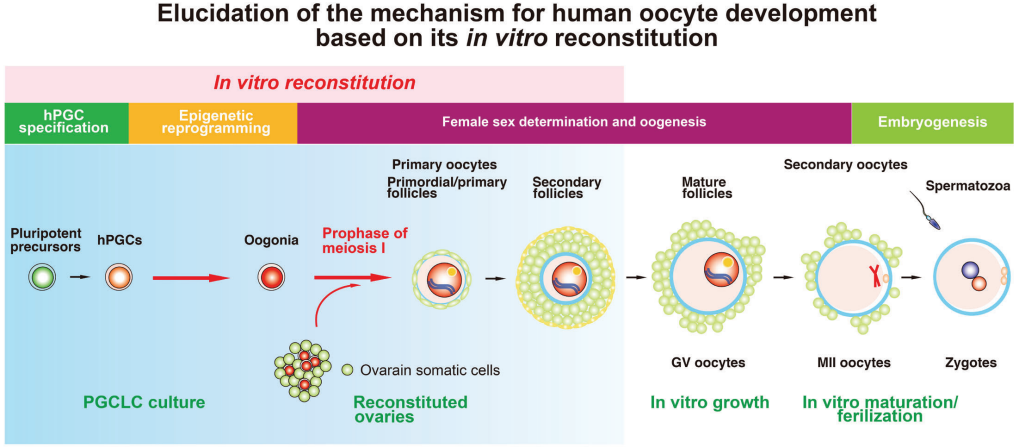


Figure 1. A scheme for human oocyte development and its *in vitro* reconstitution. This research aims to induce oocytes at early ovarian follicle stages from hiPSCs.

## Expected Research Achievements

The investigations into the mechanism of mammalian germ-cell development have been performed primarily using the mouse as a model organism. On the other hand, recent studies have shown that the mechanisms of human germ-cell development are divergent in many aspects from those of the mouse. Accordingly, to understand the mechanism of human germ-cell development and to elucidate the diseased states originating from its anomalies, it is essential to perform investigations using the human materials. This research aims to achieve *in vitro* reconstitution of human oocyte development (induction of oocytes at ovarian follicle stages) and establish a foundation for understanding the mechanism of critical events associated with human oocyte development, which include epigenetic reprogramming, X-chromosome dynamics and their impacts on oocyte development, oocyte specification, meiotic recombination, and nucleome programming for totipotency, as well as the evolutionary divergence of these key processes.

Specifically, we will perform the following four lines of investigations:

1. Optimization of the induction and culture of hPGCLCs
   hPGCLCs induced from hiPSCs serve as the origin for the *in vitro* reconstitution of human oocyte development. This research aims to optimize the induction and culture of hPGCLCs to establish a robust foundation for human *in vitro* oogenesis.
2. Establishment of an *ex vivo* culture of human fetal ovaries
   This research aims to establish an optimal culture method for maturing human embryonic ovaries consisting mainly of oogonia into those with ovarian follicles (see Figure 2)(Experiments on aborted human embryos have been approved by Ethics Committee of Kyoto University).
3. Establishment of human reconstituted ovaries based on hiPSCs
   This research aims to establish a method for inducing human embryonic ovarian somatic cells from hiPSCs and to create human reconstituted ovaries with hPGCLCs to induce human ovarian follicles *in vitro*.
4. Reconstitution of the whole oogenesis under a defined condition from mESCs
   Using the mouse as a model, this research aims to create a foundation to reconstitute the whole oocyte development from mESCs under a defined condition without using embryonic ovarian somatic cells.

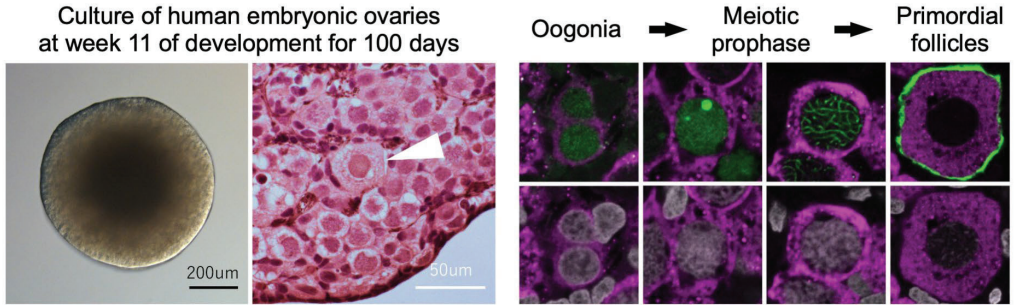


Figure 2. Culture of human embryonic ovaries (Experiments approved by Ethics Committee of Kyoto University). (left) Culture of human embryonic ovaries at week 11 of development for 100 days after dissociating them into single cells and reaggregating them as reconstituted ovaries. The arrowhead indicates the development of a primordial follicle. (right) Immunofluorescence analysis for reconstituted ovaries showing the differentiation of oogonia into primordial follicles through the meiotic prophase I.

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