Article

Current advances in artificial gametes

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Abstract

The birth of Louise Brown, the first IVF baby, in 1978 marked a breakthrough in infertility treatment. In recent decades, several important new techniques have been introduced. One limiting factor has been the requirement to use reproductive cells (gametes) for fertilization and for embryonic development. Somatic cell nuclear transfer (cloning) has been successful in mammals, opening a potential new approach for the treatment of human infertility. In addition, nuclear transfer to achieve embryo development starting from somatic cells instead of gametes, and the creation of artificial oocytes/spermatozoa has been attempted. The present paper reviews the various alternative approaches to haploidization of somatic cells.It has been observed that chromosome segregation (of the donor somatic nucleus) may take place; however, this process is largely random, thus leading to major cytogenetic abnormalities. An alternative approach is related to stem cell technology, to be further explored in the future. Culture conditions may be adjusted so that the totipotent embryonic stem cells will differentiate to specific gametes, sperm cells or egg cells. Injecting spermatozoa produced in this manner into recipient oocytes has led to pronuclear formation and early cleavage stages in some embryos. Finally, the birth of parthenogenetic mice indicates that some of these epigenetic problems can be overcome, and that some of the embryos may survive to birth.

Keywords: *artificial gamete, haploidization, nuclear transfer, stem cell*

Introduction

Advances in infertility treatment had the most extraordinary breakthrough with the birth of Louise Brown in 1978. Soon, efficiency of IVF treatment had increased dramatically with the introduction of gonadotrophins, which helped to achieve multiple follicular growth during ovarian stimulation. Yet another step was taken, a little more than a decade ago, when intracytoplasmic sperm injection (ICSI) was applied successfully. With the use of the ICSI technique combined (when necessary) with testicular/epididymal sperm aspiration, most couples with male factor related infertility could achieve pregnancy. At the present time, patients with absent gametes or gonads represent the final frontier for infertility treatment. However, another condition, poor quality (or non-viable) oocyte production, typically related to the reproductive age of the woman, poses an everyday challenge for infertility treatment. This latter group of patients account today for the majority of insoluble cases, and motivate scientists in the reproductive field to devise novel procedures.

Hence, many investigators are looking for alternative sources of spermatozoa and oocytes for infertile couples who do not have (viable) gametes. In recent years, nuclear transfer and embryonic stem cell differentiation techniques have opened new opportunities, real or theoretical, to 'generate' gametes from somatic cells or embryonic stem cells, termed somatic cell haploidization or in-vitro generated gametes.

Theoretical considerations

Cell division is a highly regulated process, and there are two distinct ways in which it takes place. One process is mitotic division, which relates to somatic cells, and the resulting

daughter cells maintain the original diploid content; the second is meiotic division, which is typical only for gametes, resulting in haploid cells. To ensure accurate meiotic chromosome segregation, the following events should be completed. First, reciprocal recombination takes place between homologues with creation of chiasmata, which allows homologues to align firmly on the metaphase I (MI) spindle. Second, there is kinetochore attachment on the meiotic spindle. Kinetochores attach to microtubules radiating from the same pole (coorientation) during MI and to microtubules originating from opposite poles (biorientation) during meiosis II (MII). Third, and finally, cohesin complexes, which keep sister chromatids together, are gradually diminished in meiosis. Cohesins are removed from chromosome arms during MI to facilitate the first round of chromosome segregation, during which homologues are segregated. Loss of cohesins around centromeres initiates the second round of chromosome segregation, during which sister chromatids separate (Martin-Lluesma *et al*., 2002; Crackower *et al*., 2003; Marston *et al*., 2004; Pawlowski *et al*., 2004). Knowledge of the temporal and spatial pattern of the expressed gene products that initially determine the type of cell division and later that are governing those processes are immensely lacking. It may not be excluded that factors associated with the meiotic process would be retained in the gamete's cytoplasm (ooplasm/ooplast) at a later stage of the progression, and when combined with an earlier stage of (somatic origin) nucleus (karyoplast), it may guide it through a division process resembling more a meiosis than a mitosis. Clearly, there are numerous other considerations in this exchange process; however, flexibility of the biological systems may be investigated in pertaining studies (Takeuchi and Palermo, 2004; Tian, 2004).

Somatic cell haploidization; study designs and results

Somatic cell haploidization is defined as a process whereby a somatic cell (2N) undergoes induced meiosis, during which the diploid chromosomes are reduced to haploid (1N). Subjecting cells to this process has the potential to generate artificial gametes (1N) from somatic cells for the potential use of infertility treatment. In theory, haploidization can be achieved by introducing a diploid somatic cell into a cytoplasm that is preprogrammed to undergo meiosis. Mammalian oocytes are ideally suited for use as a ploidy reduction machinery, because they are easily obtained and manipulation techniques are well established. To date, successful haploidization has only been achieved when the donor cells are germ cells with 2N and either 2C or 4C. First, Kimura and Yanagimachi (1995) demonstrated that injection of mouse secondary spermatocytes (2N and 2C) into MII oocytes can provide functional male gametes. Subsequently, Ogura *et al*. (1998) and Sasagawa *et al*. (1998) reported that when single spermatocyte nuclei were brought into egg cytoplasm at metaphase of MII, the spermatocyte nuclei transformed into an MI configuration, resulting in the formation of oocytes with both maternal (MII) and paternal (MI) chromosome complements. After activation, half of each chromosome set was extruded into polar bodies. Transfer of nuclei from polar bodies of 'paternal' origin into other MII oocytes resulted in the formation of oocytes with two sets of MII chromosomes, one maternal and one paternal in origin. When activated, two pronuclei and two polar bodies were formed and zygotes began development.

Additionally, normal offspring in mice were reported following use of the first polar body (PB) as a replacement for the egg nucleus, through normal fertilization by injection of spermatozoa (Wakayama and Yanagimachi, 1998). Despite the success in using germ cells (or the first polar body) to show that haploidization is possible, successful generation and fertilization of artificially haploidized gametes from somatic cells has not been reported. Whether the accurate segregation of chromosomes is sustained during somatic cell haploidization becomes the fundamental question, because improper segregation during meiosis can lead directly to embryo deaths or birth defects. Therefore, the key step to making somatic cell haploidization work is to guarantee the meiotic separation of somatic chromosomes without error.

There are primarily three different strategies that can potentially achieve somatic haploidization. First, G_0/G_1 somatic cells (2N and 2 complements of DNA or 2C) can be subjected to the cytoplasm of oocytes arrested at metaphase II (MII). After oocyte activation and release of one polar body (PB), the ploidy of the somatic cell–oocyte complex can be reduced to 1C and 1N (**Figure 1**). Second, G_0/G_1 somatic cells (2C and 2N) can be subjected to germinal vesicle (GV) stage oocytes that are arrested at the G_2/M stage of the cell cycle. After in-vitro maturation (IVM) and release of one PB, the chromosomes of somatic cells are reduced to 1C and 1N (**Figure 1**). Third, the GV oocyte can be used to reduce the chromosome number of a G_2/M phase arrested somatic cell (4C and 2N) by two rounds of reduction division. After maturation, the somatic cell–GV oocyte can release one polar body, thereby reducing the chromosome number to 2C or 1N. After activation, the oocyte can release a second polar body and reduce the chromosome to 1C and 1N (**Figure 1**). Due to theoretical considerations, because an efficient way of haploidization was not expected to be found, strategies one and two were not tested extensively (Chang *et al*., unpublished observations). It was the third strategy that was explored more in detail. To enrich the proportion of cells at G_2/M phase, mouse skin fibroblast cells were cultured with 0.5 μg/ml nocodazole for 3 h (treatment 1) or 0.25 μg/ml nocodazole for 18 h (treatment 2) in DMEM with 10% fetal calf serum before nuclear transfer. After a preliminary analysis by fluorescence-activated cell sorter (FACS) scan cytometer of treated somatic cells, large cells (25–30 μm – G_2/M phase) were transferred into the enucleated mouse GV ooplasts by micromanipulation and electrofusion. The nuclear transferred oocytes were in-vitro matured in human tubular fluid with 20% FBS for 17 h, and polar body extrusion was examined. Matured oocytes were then subjected to chemical activation. Six hours after activation, the oocytes were fixed and stained with Hoechst 33342 for investigation of nuclear configuration. To investigate the chromosomes and microtubule networks, the oocytes were fixed and stained with propidium iodide and FITC-conjugated anti-α-tubulin antibody at 4–6 h (expected MI stage) and 17 h (expected MII stage) of IVM and examined using laser scanning confocal microscopy (Chang *et al*., 2004).

Cell cycle analysis of fibroblasts in the treatment 1 group revealed that 25.28% of total cells were at G_2/M stage, and 52.77% in the treatment 2 group were at G_2/M stage. The nuclear transfer results were as follows. In the first treatment group, 56% (23/41) of the oocytes extruded the polar body after IVM (**Figure 2a**) and 48% (11/23) of them presented two pronuclei (**Figure 2b**). At the same time, in the second treatment group, 75% (18/24)