Ovarian follicle culture: advances and challenges for human and nonhuman primates

Evelyn E. Telfer, Ph.D., F.S.B.,[a,b] and Mary B. Zelinski, Ph.D.[c,d]

[a] Institute of Cell Biology, and [b] Centre for Integrative Physiology, University of Edinburgh, Edinburgh, United Kingdom; [c] Division of Reproductive and Developmental Sciences, Oregon National Primate Research Center, Beaverton; and [d] Department of Obstetrics and Gynecology, Oregon Health and Science University, Portland, Oregon

The removal and cryostorage of ovarian cortical biopsies is now offered as a fertility preservation option for young women. The only available option to restore fertility using this tissue is by transplantation, which may not be possible for all patients. The full potential of this tissue to restore fertility could be achieved by the development of in vitro systems that support oocyte development from the most immature stages to maturation. The techniques of in vitro growth (IVG) combined with in vitro maturation (IVM) are being developed with human tissue, but comparing different systems has been difficult because of the scarcity of tissue so nonhuman primates are being used as model systems. There are many challenges to developing a complete culture system that would support human oocyte development, and this review outlines the approaches being taken by several groups using tissue from women and nonhuman primate models to support each of the stages of oocyte development. (Fertil Steril® 2013;99:1523–33. ©2013 by American Society for Reproductive Medicine.)

Key Words: Follicle culture, human oocytes, primordial follicle, primates, in vitro growth

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The development of culture systems with the aim of growing oocytes from the earliest stage of follicle through to maturity for in vitro fertilization (IVF) could have a lasting impact on clinically assisted reproduction, particularly for the growing number of women who are surviving cancer only to face infertility as a result of the gametotoxic effects of cancer therapies (1). Recent success with ovarian tissue transplantation (2–4) has expanded interest in and efforts to cryopreserve and store ovarian tissue for future fertility. Ovarian tissue cryopreservation is the only option available for young patients without partners or who cannot undergo ovarian stimulation for oocyte/embryo cryopreservation and for patients for whom the risk for reintroduction of malignant cells precludes transplantation. Thus, the need for follicle culture systems that can efficiently use all classes of ovarian follicles, derived from clinically cryopreserved ovarian tissue, as sources of gametes would maximize reproductive potential for future fertility. However, complete in vitro growth (IVG) from immature primordial stages with subsequent IVF of oocytes followed by embryo transfer and production of live offspring has, so far, been achieved only in mice (5, 6). Several groups have focused on culturing later stages of follicle development from rodents and have produced developmentally competent oocytes and viable offspring (7–11). The success of these techniques has encouraged the demanding challenge of adapting them for humans and other primates.

Although these techniques have been used to study the regulation of follicle development, the ultimate aim of follicle culture is to increase the availability of developmentally competent oocytes from what would be available from conventional methods, and there is still much to do before follicle culture can be used as a strategy for obtaining competent oocytes. In recent years a great deal of progress has been made in developing culture techniques

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Reprint requests: Evelyn E. Telfer, Ph.D., F.S.B., Centre for Integrative Physiology, University of Edinburgh, Hugh Robson Building, George Square, Edinburgh, EH8 9XE United Kingdom (E-mail: evelyn.telfer@ed.ac.uk).

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for humans and nonhuman primates, and in this review we describe the technologies and discuss the prospects as well as the problems of applying them clinically.

FOLLICULAR DEVELOPMENT

Female reproductive function requires cyclic development and maturation of ovarian follicles on a background of continuous activation from the pool of primordial follicles. Primordial follicles are formed before birth and represent a population of germ cells from which recruitment for growth occurs throughout the woman's reproductive life. Follicular growth and development involves a series of complex and precisely regulated events: 1) initiation of primordial follicle growth and development to the preantral follicle stage; 2) formation of antral follicles where expansion to the preovulatory or graafian follicle is associated with granulosa cell proliferation and antral fluid accumulation within the basement membrane; and 3) rupture of the graafian follicle, releasing a cumulus-oocyte complex at ovulation in response to the midcycle LH surge (Fig. 1A).

As the oocyte grows within the follicle it is held in meiotic arrest at the dictyate stage of prophase I, but during development within the follicle it must acquire the ability to resume meiosis (meiotic competence) and the ability to support fertilization and embryonic development (developmental competence). Thus the oocyte depends on the local environment within the follicle for subsequent function as a gamete, and the formation and maintenance of connections facilitating bidirectional communication between the oocyte and granulosa cells are key to oocyte development in all species.

The development of culture conditions for immature germ cells (both eggs and sperm) is one of the greatest technical challenges of reproductive technology. An understanding of the physiologic requirements of the oocyte, granulosa, theca, and perhaps even the stromal cells is needed. These requirements are complex and change during growth, so a major consideration is the starting point of the culture system, i.e., which stage of follicle to start with. The majority of follicles within the ovary in all young mammalian females are at the primordial stage of development, and those follicles are continually being used during reproductive life (12). We do not know if this pool represents a homogeneous population but it is thought that at this stage follicles have not yet been exposed to selection processes that lead to follicle degeneration (13, 14). Although rodents are excellent models for pioneering technologies, intermediate species are needed to test feasibility for human applications. Follicles of some domestic animals (cows, sheep, and goats) can resemble those of humans regarding growth rates and size, but the protracted length of folliculogenesis in vivo, estimated in women to be 90 days from the entrance of a preantral follicle into the growing pool to preovulatory follicles (15), and the long length of the follicular phase of the spontaneous menstrual cycle in nonhuman primates (~2 weeks compared with a few days in domestic mammals) more closely reflect that of women. Therefore, nonhuman primates are emerging as an important translational model to advance technologic developments in follicle culture.

DEVELOPMENT OF FOLLICLES IN VITRO

Several approaches have been taken to develop human follicles in vitro with the use of fresh (16, 17) and thawed cryopreserved (17, 18) human cortical tissue. It is now clear that if we are to achieve complete development of human oocytes, a dynamic multistep culture system is required to support each of the transitional stages (16, 19–21). The first step is to support the initiation of primordial follicle development and early growth, the second stage is to optimize the growth of follicles from preantral to antral stages, with the completion of oocyte growth being achieved during the third stage. The focus should be primarily on oocyte development, which may not require the development of large follicular structures but rather the maintenance of differentiated somatic cells in contact with the developing oocyte. A multistep system for IVG follicles has been proposed (22, 23) to produce competent oocytes from human ovarian cortical tissue. The multistep approach needs to support the changing requirements of the developing oocyte and its surrounding somatic (granulosa) cells with the main focus being on maintaining oocyte–somatic cell interactions. Several groups have worked on each of the steps required to support human oocyte development in vitro: 1) activation of primordial follicles through culturing ovarian cortex (16, 17); 2) isolation and culture of growing preantral follicles to achieve oocyte growth and development (16, 24–31); and 3) aspiration and maturation of oocyte-cumulus complexes (32, 33). The aim of ongoing research in this field has been to combine each of these steps to achieve complete development of human oocytes (16). Progress in achieving this goal and the use of nonhuman primate models will be reviewed.

Activation of Primordial Follicles

The majority of follicles within ovarian cortical tissue are quiescent primordial, so the first consideration of an IVG system should be to optimize initiation of primordial follicles in vitro and support early follicle development. The factors regulating follicle initiation and early growth are still not well defined, but the process requires a combination of inhibitory, stimulatory, and maintenance factors (34). Studies using knockout mouse models have demonstrated the importance of the phosphatidylinositol-3′-kinase (PI3K)–Akt signaling pathway within the oocyte in regulating follicle activation (35). The phosphatase and tensin homologue deleted on chromosome ten (PTEN) acts as a negative regulator of this pathway and suppresses initiation of follicle development (35). The transcription factor FOXO3a is a downstream effector of this pathway and acts to inhibit follicle recruitment (36). However, primordial follicles of fetal and juvenile macaque ovaries lack FOXO3a expression, suggesting that alternative transcription factors may mediate follicle activation in primates (37). Other components of this pathway depend on the mammalian target of rapamycin complex 1 (mTORC1), a serine/threonine kinase that regulates cell growth and proliferation in response to growth factors and nutrients and regulates primordial follicle activation (38). How these pathways regulate human follicle