CHAPTER 8

Maturation of primordial follicles – the next step

Julius Hreinsson, Inger Britt Carlsson, and Outi Hovatta

INTRODUCTION

The survival rates of patients after treatment against malignant disease have dramatically increased during the last few decades, primarily due to therapeutic improvements[1]. One of the possible side-effects of such treatments, however, is the loss of fertility, which can seriously affect the adult lives of survivors. Options for fertility preservation for men through cryopreservation and storage of sperm have been routinely available since the 1970s and are now considered an essential part of any comprehensive cancer care program[2]. The possibilities for women, however, have traditionally been more limited.

The gonadotoxic effect of chemotherapy and radiotherapy is well established; it is dose dependent and varies according to regimens used. A reduction in follicle numbers is seen[3] and the course of ovarian dysfunction is consistent with the destruction of a fixed number of follicles[4]. Temporary or permanent cessation of menstruation may follow therapy and a greatly elevated rate of premature menopause is found in women receiving treatment against malignancy[5]. Survivors of cancer in childhood have been shown to have a diminished ovarian reserve in spite of regular menstrual cycles and may have a shortened reproductive life span and early menopause[6]. Radiotherapy causes destruction of the oocytes and reduction of the follicular reserve. Total body irradiation and high dose chemotherapy, for example before bone marrow transplantation, can be expected to destroy almost all of the oocytes in the ovaries of a female patient[7,8].

Strategies for preservation of fertility for women include cryopreservation of embryos, mature or immature oocytes, or follicles in ovarian cortical tissue. The first two options are not always possible, especially for young women and girls, since in-vitro fertilization (IVF) treatment is a prerequisite to obtain mature oocytes. The number of oocytes obtained at oocyte retrieval is limited at any age. Immature follicles are the largest proportion of the ovarian reserve and fertility preservation through cryopreservation of ovarian cortical biopsies is now offered in many of the larger university hospitals. This is a practical way for storing the large number of oocytes present in immature ovarian follicles. Patients with predicted premature menopause or Turner's syndrome may also benefit from these methods[9].

Good survival of human ovarian follicles in biopsied ovarian cortical tissue has been shown after cryopreservation and thawing using histologic evaluation, transmission electron microscopy, fluorescent viability markers, organ culture, and xenotransplantation to immunodeficient

08-Human Oocytes-chapter08-ppp.i137 137 14/9/06 16:21:32

mice[10–16]. Live born individuals after cryopreservation and thawing of ovarian tissue followed by autotransplantation were first observed in experimental animals. This procedure has been successful in mice[17–21], sheep[22,23], and various other animals[24–27].

Developmental potential of the follicles and oocytes in thawed tissue has also been shown in humans by autotransplantation of thawed ovarian tissue. Evidence of follicular function following the procedure includes follicular development induced by follicle-stimulating hormone (FSH) stimulation, estradiol production, and ovulation[28]. Follicular and endometrial development have been observed[29] and hormonal analysis has shown a temporary decrease in FSH levels and estradiol production[30]. Oocyte collection and attempted IVF have also been reported after such a procedure[31].

Recently, two reports, one of a live born child and another of an ongoing pregnancy in human using this procedure, have been published[32,33], confirming the clinical motivation for cryopreservation of ovarian tissue. In survivors of cancer treatment there is a potential danger of re-introducing the original disease to the patient when autografting thawed tissue since malignant cells may reside in the tissue[34]. This depends on the type of cancer the patient had. This possible risk of re-transmission of cancer through autotransplantation of thawed tissue may be greatest in those patients who need fertility preservation most, for example patients with blood borne diseases such as leukemia. For these patients, culture from primordial to antral stages followed by oocyte maturation in vitro is probably the only safe alternative to obtain fertilizable oocytes.

FOLLICLE DEVELOPMENT

Ovarian follicles are formed as closed compartments consisting of an oocyte surrounded by a single layer of granulosa cells enclosed by a basal membrane. As follicles grow, the oocyte increases in size and the granulosa cells multiply in numbers. Formation of an antral cavity precedes ovulation and final oocyte maturation.

Culture of follicles in ovarian cortical tissue is a complex procedure. The whole process of in-vitro growth and maturation from the primordial stage has, until now, only been successful with live born pups in the mouse[35,36]. Using later stage follicles, also from mice, developmentally competent mature oocytes and live born young have been obtained[37–39]. In the human, the time for follicle growth from initiation until ovulation is a much longer process than in rodents. It has been estimated to take up to 200 days[40], although this may be an overestimation.

The early stages of follicle growth depend on a multitude of factors, including FSH for which human primary follicles already have receptors[41]. FSH acts as a survival factor and promotes the growth of the early follicles in vitro[42]. A number of growth factors which influence follicle growth and development have also been identified in recent years. Among these are the members of the TGF-β superfamily of growth factors, shown to be important at these early stages[43]. Of these, anti-Müllerian hormone (AMH, also known as Müllerian inhibiting substance or MIS) inhibits the initiation of growth of the primordial follicles both in human[44] and rat[45], although in 4-week cultures it has also been reported to promote the growth of the follicles[46]. Activins, inhibins, growth differentiation factor 9 (GDF-9), GDF-9B, and the bone morphogenetic proteins or BMPs have functions in follicle development have functions in various species, including humans[46–48]. Other factors, such as kit ligand and stem cell factor, have also been shown to affect early follicle growth in the human[49,50].

During growth the follicles migrate into the medullar region of the ovary. Gonadotropins, especially FSH, are of primary importance for follicular growth and they sustain follicular steroidogenesis. According to the ‘two-cell, two-gonadotropins’ theory, theca interstitial cells are stimulated by LH to produce aromatizable

08-Human Oocytes-chapter08-ppp.i138 138 14/9/06 16:21:33

androgens that are transported to the granulosa cells where they are converted to estrogens by aromatizing enzymes which are induced by FSH[51]. When the follicles have reached a size of 2 mm, they become more dependent on FSH for growth and the steroid production increases. When the middle part of the menstrual cycle approaches, there is a dramatic increase in estrogen, followed by an LH and to a lesser extent an FSH surge. This triggers the dominant follicle to ovulate[52]. After ovulation, the follicle reorganizes to become the corpus luteum, responsible for progesterone production and early maintenance of pregnancy.

COLLECTION OF OVARIAN TISSUE

Human ovarian tissue for research purposes is difficult to obtain. Ovarian biopsies may be collected during laparoscopic surgery, such as sterilizations or other gynecological operations. We have also obtained small ovarian biopsies ($2 \times 2 \times 5$ mm) during cesarean sections with good results[53]. For cryostorage of primordial follicles, a biopsy specimen from one ovary, one-fifth of the ovary or less, contains large numbers of follicles, especially in younger patients with good ovarian reserve.

To minimize apoptosis in the tissue, the biopsy is collected in warm Hepes-buffered culture medium, transported immediately to the laboratory, and processed for culture without delay. Whether the biopsy should be kept on ice until processing, or kept at physiologic temperature, has been the subject of some research[54,55]. When preparation is performed after any length of time, a cold collection medium may be recommended[56].

CULTURE OF OVARIAN CORTICAL TISSUE

The high density of the stroma in human ovarian cortical tissue has contributed to the delay in developing a successful system for isolating follicles. Enzymatic isolation of preantral follicles has been successful using human ovarian cortical tissue, where culture was performed for up to 5 days. In these follicles FSH was seen to induce follicular DNA synthesis, antrum formation in larger follicles, and estradiol production[57]. Culture of isolated human primordial follicles for over 24 h, however, has not been successful[58]. In a comparative study of isolated and non-isolated follicles, significantly better survival was seen for follicles cultured within tissue slices than among partially isolated follicles[59].

In organ culture, extracellular matrix has been shown to be beneficial for the survival of the follicles[14]. An immediate activation of growth of the primordial follicles has been observed, with a majority of the follicles leaving the latent primordial stage after one week of culture[59]. Using this culture system, follicle growth up to the secondary stage regularly occurs, and antral follicles are occasionally observed[14,59]. Also follicles in frozen-thawed ovarian tissue grow in such cultures[14]. It is important to use carefully tested freezing protocols[60,61]. In our studies, the optimal size of the pieces in culture was 1–2 mm^3. Cubes of tissue were better than thin slices $1 \times 1 \times 4$ mm due to the increased surface area for nutrient and waste exchange[62].

Several factors have been identified that are important for growth of the follicles in vitro. FSH was seen to reduce atresia and to increase the mean diameter of healthy follicles in organ culture. HSA supplemented with insulin/transferrin/selenium mix was also beneficial compared with serum[42]. Insulin and insulin-like growth factors I (IGF-I) and IGF-II have also been seen to improve survival of follicles and to increase the proportion of primary follicles after 2 weeks of culture[63]. GDF-9 significantly promoted the growth and survival of follicles in culture[53]. Due to progressive atresia of the follicles during long-term culture, we have investigated whether apoptosis can be prevented by adding 8-bromo-cyclic adenosine monophosphate (AMP) or

08-Human Oocytes-chapter08-ppp.i139 139 14/9/06 16:21:33

guanosine monophosphate (GMP)[64,65]. On the basis of these studies we now regularly add cGMP as an apoptosis inhibitor to the culture medium[64]. Table 8.1 shows the hormones and growth factors that we have studied in this culture model and their effects, while other media constituents are shown in Table 8.2. In Figure 8.1a, a primordial follicle before culture is shown, while Figure 8.1b shows a primary follicle after 1 week of culture. After 2 weeks in culture (Figure 8.1c) and 4 weeks in culture (Figure 8.1d) the follicles are mostly secondary.

We are now using a culture medium which is composed of α-minimal essential medium, with added human serum albumin (5 mg/ml), 1% ITS (insulin, transferrin, selenium mixture for culture supplements), FSH (0.5 IU/ml), GDF-9 (various concentrations have been tested), cGMP (1.1 mg/ml), and antibiotic/antimycotic solution. We have been successful in culturing viable follicles for up to 6 weeks, at which stage the majority of the follicles are at secondary stage (Carlsson et al., unpublished). As we proceed with our work, we are testing new compo-

Table 8.1 Hormones and growth factors which our group has studied using ovarian cortical tissue organ culture of slices/cubes in extracellular matrix and the effect(s) observed

Supplement	*Dose(s)*	*Length of culture*	*Results*	*Reference*
rrGDF-9	200 ng/ml	14 days	• Increase in follicle activation and development • Decrease in atresia	Hreinsson et al., 2002[53]
8-br-cGMP	5 mM	14 days	• Increase in secondary follicles • Increase in E_2 • Decrease in atresia	Scott et al., 2004[64]
8-br-cAMP	0.5 mM	21 days	• Increase in secondary follicles • Decrease in atresia	Zhang et al., 2004[65]
rhSCF	1, 10, 100 ng/ml	14 days	• No effect on follicle growth	Carlsson et al., 2005[50]
monoclonal anti-c-*kit* antibody	800 ng/ml	7–14 days	• Rapid atresia of primordial oocytes	Carlsson et al., 2005[50]
rrAMH	10, 30, 100, 300 ng/ml	7 days	• Decrease in follicle activation • Decrease in atresia (at 100 ng/ml)	Carlsson et al., 2005[44]

Table 8.2 Media supplements and concentrations

Supplement	*Dose*	*Effect*
α-Minimal essential medium	N/A	• Serum free base
Human serum albumin	5 mg/ml	• Protein component
Insulin/transferrin/selenium	1%	• Growth supplements
Antibiotic/antimycotic	1%	• Contamination preventative
rhFSH	0.5 IU/ml	• Growth and maturation promoter

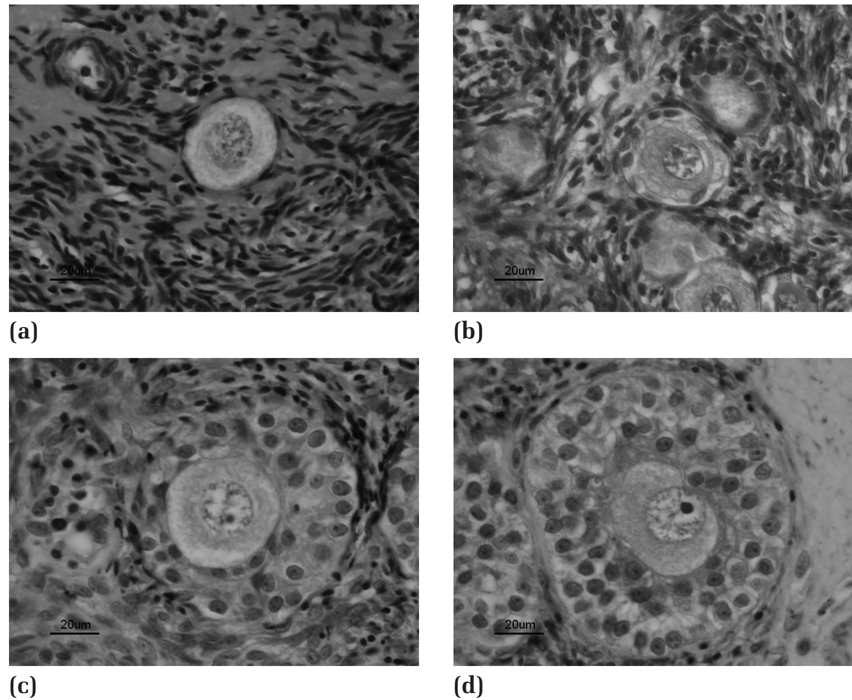

Figure 8.1 (a) Primordial follicle before culture; (b) primary follicle after 1 week of culture with GDF-9 at 200 ng/ml; (c) secondary follicle after 2 weeks of culture with GDF-9 at 200 ng/ml; (d) secondary follicle after 4 weeks of culture with GDF-9 at 200 ng/ml

sitions of the medium to improve antral follicle development. Antral follicles are a prerequisite for obtaining and removing the cumulus–oocyte complex for final maturation of the oocyte in vitro. The maturation of human primordial oocytes to metaphase II fertilizable oocytes is a long and complicated process, and much research is required before a reliable clinical procedure can be realized.

GOAL OF THESE TECHNIQUES – CURRENT PERSPECTIVES

The possibility of obtaining mature oocytes through in-vitro culture of follicles in ovarian tissue would benefit several patient groups. Women and girls having ovarian tissue cryopreserved to preserve their fertility prior to potentially sterilizing cancer treatment will need this technique to obtain fertilizable oocytes from the thawed tissue. Many of these survivors of cancer have been cured of blood borne malignancies precluding re-transplantation of the tissue for in-vivo development. Current techniques for re-transplantation of thawed ovarian tissue for other patients needing fertility preservation are not yet optimal. This includes patients with rheumatic diseases, premature menopause, Turner's syndrome, and localized malignancies. In-vitro culture allows closer observation of the follicles and potentially a better yield of oocytes from small follicles. With better understanding of follicle growth and oocyte maturation, these

08-Human Oocytes-chapter08-ppp.i141 141 14/9/06 16:21:34

methods could also benefit women with certain gene defects, such as an inactivating mutation in the FSH receptor[66].

There is a great need to focus research on developing these techniques for human ovarian tissue due to a rapid accumulation of patients waiting to utilize cryopreserved ovarian cortical tissue. This follicle culture method offers us an excellent model for understanding the physiology of the oocyte, follicle recruitment, and development. The role of growth factors can also be critically examined using this method. If successful, this technique might also be beneficial for stem cell research as a source of oocytes for nuclear transfer. The ability to obtain large numbers of oocytes from ovarian tissue biopsies is a prerequisite for somatic cell nuclear transfer and stem cell derivation.

SUMMARY

Ovarian cortical tissue contains the vast majority of all oocytes. Culture of human ovarian cortical tissue to obtain mature fertilizable oocytes would be beneficial for young girls and women who have cryopreserved ovarian tissue and can be predicted to lose their oocytes due to chemotherapy, radiotherapy, or genetic causes. Further, it would be particularly important for women at risk of relapse of the malignancy after transplantation of frozen-thawed tissue, not to mention women with hematologic and ovarian malignancies. In the mouse, live offspring have been born after culture from primordial ovarian follicles, but in humans the process of follicular maturation is much longer and has not yet resulted in offspring. It has been shown that isolated human primordial follicles do not grow in culture. Within pieces of fresh and frozen-thawed human ovarian cortical tissue on extracellular matrix, follicles can be grown for many weeks. They regularly reach the secondary stage and occasionally the antral stage.

Several hormones have been shown to promote survival and growth of early follicles in vitro. We are currently adding FSH, insulin, GDF-9, and cGMP. Regular development to the antral follicle stage would be needed for cumulus-enclosed oocytes to be removed from the follicle for further in-vitro maturation (IVM) of the oocytes. The culture system has to be further developed before oocytes from primordial follicles can be used clinically.

REFERENCES

1. McVie JG. Cancer treatment: the last 25 years. Cancer Treat Rev 1999; 25: 323–31.
2. Kelleher S, Wishart SM, Liu PY et al. Long-term outcomes of elective human sperm cryostorage. Hum Reprod 2001; 16: 2632–9.
3. Nicosia SV, Matus-Ridley M, Meadows AT. Gonadal effects of cancer therapy in girls. Cancer 1985; 55: 2364–72.
4. Howell S, Shalet S. Gonadal damage from chemotherapy and radiotherapy. Endocrinol Metab Clin North Am 1998; 27: 927–43.
5. Byrne J, Fears TR, Gail MH et al. Early menopause in long-term survivors of cancer during adolescence. Am J Obstet Gynecol 1992; 166: 788–93.
6. Larsen EC, Muller J, Rechnitzer C et al. Diminished ovarian reserve in female childhood cancer survivors with regular menstrual cycles and basal FSH <10 IU/L. Hum Reprod 2003; 18: 417–22.
7. Wallace WH, Shalet SM, Crowne EC et al. Ovarian failure following abdominal irradiation in childhood: natural history and prognosis. Clin Oncol (R Coll Radiol) 1989; 1: 75–9.
8. Wallace WH, Thomson AB, Kelsey TW. The radiosensitivity of the human oocyte. Hum Reprod 2003; 18: 117–21.
9. Hreinsson JG, Otala M, Fridstrom M et al. Follicles are found in the ovaries of adolescent girls with Turner's syndrome. J Clin Endocrinol Metab 2002; 87: 3618–23.

08-Human Oocytes-chapter08-ppp.i142 142 14/9/06 16:21:35

10. Hovatta O, Silye R, Krausz T et al. Cryopreservation of human ovarian tissue using dimethylsulphoxide and propanediol-sucrose as cryoprotectants. Hum Reprod 1996; 11: 1268–72.

11. Gook DA, Edgar DH, Stern C. Effect of cooling rate and dehydration regimen on the histological appearance of human ovarian cortex following cryopreservation in 1, 2-propanediol. Hum Reprod 1999; 14: 2061–8.

12. Oktay K, Nugent D, Newton H et al. Isolation and characterization of primordial follicles from fresh and cryopreserved human ovarian tissue. Fertil Steril 1997; 67: 481–6.

13. Cortvrindt RG, Smitz JE. Fluorescent probes allow rapid and precise recording of follicle density and staging in human ovarian cortical biopsy samples. Fertil Steril 2001; 75: 588–93.

14. Hovatta O, Silye R, Abir R et al. Extracellular matrix improves survival of both stored and fresh human primordial and primary ovarian follicles in long-term culture. Hum Reprod 1997; 12: 1032–6.

15. Gook DA, Edgar DH, Borg J et al. Oocyte maturation, follicle rupture and luteinization in human cryopreserved ovarian tissue following xenografting. Hum Reprod 2003; 18: 1772–81.

16. Schmidt KL, Byskov AG, Nyboe Andersen A et al. Density and distribution of primordial follicles in single pieces of cortex from 21 patients and in individual pieces of cortex from three entire human ovaries. Hum Reprod 2003; 18: 1158–64.

17. Parrott DMV. The fertility of mice with orthotopic ovarian grafts derived from frozen tissue. J Reprod Fertil 1960; 1: 230–41.

18. Gunasena KT, Villines PM, Critser ES et al. Live births after autologous transplant of cryopreserved mouse ovaries. Hum Reprod 1997; 12: 101–6.

19. Sztein J, Sweet H, Farley J et al. Cryopreservation and orthotopic transplantation of mouse ovaries: new approach in gamete banking. Biol Reprod 1998; 58: 1071–4.

20. Liu J, Van der Elst J, Van den Broecke R et al. Live offspring by *in vitro* fertilization of oocytes from cryopreserved primordial mouse follicles after sequential *in vivo* transplantation and *in vitro* maturation. Biol Reprod 2001; 64: 171–8.

21. Snow M, Cox SL, Jenkin G et al. Generation of live young from xenografted mouse ovaries. *Science* 2002; 297: 2227.

22. Gosden RG, Baird DT, Wade JC et al. Restoration of fertility to oophorectomized sheep by ovarian autografts stored at –196 degrees C. Hum Reprod 1994; 9: 597–603.

23. Salle B, Demirci B, Franck M et al. Normal pregnancies and live births after autograft of frozen-thawed hemi-ovaries into ewes. Fertil Steril 2002; 77: 403–8.

24. Wang X, Chen H, Yin H et al. Fertility after intact ovary transplantation. Nature 2002; 415: 385.

25. Candy CJ, Wood MJ, Whittingham DG. Follicular development in cryopreserved marmoset ovarian tissue after transplantation. Hum Reprod 1995; 10: 2334–8.

26. Aubard Y, Newton H, Scheffer G et al. Conservation of the follicular population in irradiated rats by the cryopreservation and orthotopic autografting of ovarian tissue. Eur J Obstet Gynecol Reprod Biol 1998; 79: 83–7.

27. Gunasena KT, Lakey JR, Villines PM et al. Antral follicles develop in xenografted cryopreserved African elephant (*Loxodonta africana*) ovarian tissue. Anim Reprod Sci 1998; 53: 265–75.

28. Oktay K, Karlikaya G. Ovarian function after transplantation of frozen, banked autologous ovarian tissue. N Engl J Med 2000; 342: 1919.

29. Radford JA, Lieberman BA, Brison DR et al. Orthotopic reimplantation of cryopreserved ovarian cortical strips after high-dose chemotherapy for Hodgkin's lymphoma. Lancet 2001; 357: 1172–5.

30. Callejo J, Salvador C, Miralles A et al. Long-term ovarian function evaluation after autografting by implantation with fresh and frozen-thawed human ovarian tissue. J Clin Endocrinol Metab 2001; 86: 4489–94.

31. Tryde Schmidt KL, Yding Andersen C, Starup J et al. Orthotopic autotransplantation of cryopreserved ovarian tissue to a woman cured of cancer – follicular growth, steroid production and

08-Human Oocytes-chapter08-ppp.i143 143 14/9/06 16:21:35

oocyte retrieval. Reprod Biomed Online 2004; 8: 448–53.

32. Donnez J, Dolmans MM, Demylle D et al. Livebirth after orthotopic transplantation of cryopreserved ovarian tissue. Lancet 2004; 364: 1405–10.

33. Meirow D, Levron J, Eldar-Geva T et al. Pregnancy after transplantation of cryopreserved ovarian tissue in a patient with ovarian failure after chemotherapy. N Engl J Med 2005; 353: 318–21.

34. Shaw JM, Bowles J, Koopman P et al. Fresh and cryopreserved ovarian tissue samples from donors with lymphoma transmit the cancer to graft recipients. Hum Reprod 1996; 11: 1668–73.

35. Eppig JJ, O'Brien MJ. Development *in vitro* of mouse oocytes from primordial follicles. Biol Reprod 1996; 54: 197–207.

36. O'Brien MJ, Pendola JK, Eppig JJ. A revised protocol for *in vitro* development of mouse oocytes from primordial follicles dramatically improves their developmental competence. Biol Reprod 2003; 68: 1682–6.

37. Eppig JJ, Schroeder AC. Capacity of mouse oocytes from preantral follicles to undergo embryogenesis and development to live young after growth, maturation, and fertilization *in vitro*. Biol Reprod 1989; 41: 268–76.

38. Spears N, Boland NI, Murray AA et al. Mouse oocytes derived from *in vitro* grown primary ovarian follicles are fertile. Hum Reprod 1994; 9: 527–32.

39. Cortvrindt R, Smitz J, Van Steirteghem AC. A morphological and functional study of the effect of slow freezing followed by complete in-vitro maturation of primary mouse ovarian follicles. Hum Reprod 1996; 11: 2648–55.

40. Gougeon A. Dynamics of follicular growth in the human: a model from preliminary results. Hum Reprod 1986; 1: 81–7.

41. Oktay K, Briggs D, Gosden RG. Ontogeny of follicle-stimulating hormone receptor gene expression in isolated human ovarian follicles. J Clin Endocrinol Metab 1997; 82: 3748–51.

42. Wright CS, Hovatta O, Margara R et al. Effects of follicle-stimulating hormone and serum substitution on the in-vitro growth of human ovarian follicles. Hum Reprod 1999; 14: 1555–62.

43. Erickson GF, Shimasaki S. The role of the oocyte in folliculogenesis. Trends Endocrinol Metab 2000; 11: 193–8.

44. Carlsson IB, Scott JE, Visser JA et al. Anti-Müllerian hormone inhibits initiation of growth of human primordial ovarian follicles in vitro. Hum Reprod 2006; [Epub ahead of print].

45. Visser JA, Themmen AP. Anti-Mullerian hormone and folliculogenesis. Mol Cell Endocrinol 2005; 234: 81–6.

46. Schmidt KL, Kryger-Baggesen N, Byskov AG et al. Anti-Mullerian hormone initiates growth of human primordial follicles in vitro. Mol Cell Endocrinol 2005; 234: 87–93.

47. McGrath SA, Esquela AF, Lee SJ. Oocyte-specific expression of growth/differentiation factor-9. Mol Endocrinol 1995; 9: 131–6.

48. Juengel JL, McNatty KP. The role of proteins of the transforming growth factor-beta superfamily in the intraovarian regulation of follicular development. Hum Reprod Update 2005; 11: 143–60.

49. Driancourt MA, Reynaud K, Cortvrindt R et al. Roles of KIT and KIT LIGAND in ovarian function. Rev Reprod 2000; 5: 143–52.

50. Carlsson IB, M.P.E. L, Scott JE, Louhio H et al. Kit ligand and c-kit are expressed during early human ovarian follicular development and their interaction is required for the survival of follicles in long-term culture. *Reproduction* 2006; 131(4): 641–9.

51. Hillier SG, Whitelaw PF, Smyth CD. Follicular oestrogen synthesis: the 'two-cell, two-gonadotrophin' model revisited. Mol Cell Endocrinol 1994; 100: 51–4.

52. Yen SCC, Jaffe RB, Barbieri RL. Reproductive Endocrinology, 4th edn. WB Saunders Company, Philadelphia, Pennsylvania, 1999.

53. Hreinsson JG, Scott JE, Rasmussen C et al. Growth differentiation factor-9 promotes the growth, development, and survival of human ovarian follicles in organ culture. J Clin Endocrinol Metab 2002; 87: 316–21.

08-Human Oocytes-chapter08-ppp.i144 144 14/9/06 16:21:35

54. Weissman A, Gotlieb L, Colgan T et al. Preliminary experience with subcutaneous human ovarian cortex transplantation in the NOD-SCID mouse. Biol Reprod 1999; 60: 1462–7.

55. Lucci CM, Kacinskis MA, Rumpf R et al. Effects of lowered temperatures and media on short-term preservation of zebu (*Bos indicus*) preantral ovarian follicles. Theriogenology 2004; 61: 461–72.

56. Schmidt KL, Ernst E, Byskov AG et al. Survival of primordial follicles following prolonged transportation of ovarian tissue prior to cryopreservation. Hum Reprod 2003; 18: 2654–9.

57. Roy SK, Treacy BJ. Isolation and long-term culture of human preantral follicles. Fertil Steril 1993; 59: 783–90.

58. Abir R, Roizman P, Fisch B et al. Pilot study of isolated early human follicles cultured in collagen gels for 24 hours. Hum Reprod 1999; 14(5): 1299–301.

59. Hovatta O, Wright C, Krausz T et al. Human primordial, primary and secondary ovarian follicles in long-term culture: effect of partial isolation. Hum Reprod 1999; 14: 2519–24.

60. Hovatta O. Methods for cryopreservation of human ovarian tissue. Reprod Biomed Online 2005; 10: 729–34.

61. Hreinsson J, Zhang P, Swahn ML et al. Cryopreservation of follicles in human ovarian cortical tissue. Comparison of serum and human serum albumin in the cryoprotectant solutions. Hum Reprod 2003; 18: 2420–8.

62. Scott JE, Carlsson IB, Bavister BD et al. Human ovarian tissue cultures: extracellular matrix composition, coating density and tissue dimensions. Reprod Biomed Online 2004; 9: 287–93.

63. Louhio H, Hovatta O, Sjoberg J et al. The effects of insulin, and insulin-like growth factors I and II on human ovarian follicles in long-term culture. Mol Hum Reprod 2000; 6: 694–8.

64. Scott JE, Zhang P, Hovatta O. Benefits of 8-bromo-guanosine 3′,5′-cyclic monophosphate (8-br-cGMP) in human ovarian cortical tissue culture. Reprod Biomed Online 2004; 8: 319–24.

65. Zhang P, Louhio H, Tuuri T et al. In vitro effect of cyclic adenosine 3′, 5′-monophosphate (cAMP) on early human ovarian follicles. J Assist Reprod Genet 2004; 21: 301–6.

66. Aittomaki K, Herva R, Stenman UH et al. Clinical features of primary ovarian failure caused by a point mutation in the follicle-stimulating hormone receptor gene. J Clin Endocrinol Metab 1996; 81: 3722–6.

08-Human Oocytes-chapter08-ppp.i145 145 14/9/06 16:21:36

08-Human Oocytes-chapter08-ppp.i146 146

14/9/06 16:21:36