Expression of receptors for insulin-like growth factor-I and transforming growth factor-β in human follicles

Jianping Qu, Pierre Arnaud Godin, Michelle Nisolle and Jacques Donnez

Laboratory of In Vitro Fertilization, Department of Gynecology, Hospital of Saint Luc, Catholic University of Louvain, Brussels, Belgium

1To whom correspondence should be addressed at: Department of Gynaecology, Hospital of Saint Luc, Catholic University of Louvain (UCL), Avenue Hippocrate 10, UCL 10/9502, 1200 Brussels, Belgium

The in-vitro growth of immature oocytes in early follicles from cryopreserved human ovarian tissues is a new concept in in-vitro fertilization programmes for the treatment of infertile and cancer patients. To better understand the regulatory mechanism of follicular development, immunohistochemistry was used to study the expression of insulin-like growth factor (IGF) type I receptor (IGF-IR) and transforming growth factor-β (TGFβ) type I (TβR-I) and type II (TβR-II) receptors in fresh and frozen ovarian tissues from 14 women. Immunoreactivities for IGF-IR and TβR-I were present simultaneously in the oocytes of primordial, pre-antral and antral follicles. Staining for both IGF-IR and TβR-I was also observed in granulosa cells of primordial, pre-antral and antral follicles. IGF-IR and TβR-I also stained in thecal cells of pre-antral and antral follicles. Stromal cells in surrounding ovarian tissue expressed IGF-IR and TβR-I at various follicular stages. Unlike TβR-I, TβR-II was expressed only in the oocytes of primordial and primary follicles, and with weak staining intensity in thecal cells. No significant staining for TβR-II was found in oocytes and granulosa cells of antral follicles. There was no difference in staining patterns for IGF-IR, TβR-I and TβR-II between fresh and frozen ovarian tissues, indicating that cryopreservation might not significantly alter the immunoreactivities of these receptors in frozen ovarian tissue. The results suggest that IGF-I and TGFβ may participate in the regulation of follicular growth by binding to their receptors through an autocrine or paracrine mechanism. IGF-I and TGFβ may be useful in regulating the in-vitro or in-vivo maturation of oocytes not only in later follicles but also very early follicles, from cryopreserved ovarian tissues for clinical use in the future.

Key words: cryopreservation/follicle/insulin-like growth factor receptor/transforming growth factor-β

Introduction

Cryopreservation of human ovarian tissues, in combination with the maturation of oocytes from immature follicles in vivo or in vitro, is becoming an emerging technology in in-vitro fertilization programmes (IVF) for the treatment of infertile and cancer patients (Newton et al., 1996; Oktay et al., 1998). Ovarian tissue banking is considered an alternative to the storage of frozen oocytes due to several advantages (Oktay et al., 1998). The oocytes in primordial follicles are far smaller than when fully ripened at metaphase II. They are less differentiated, possessing fewer organelles, and lacking zona pellucida and cortical granules. These characteristics are potentially beneficial for freeze preservation. Unlike fully grown oocytes, oocytes arrested in prophase should theoretically be at low risk of cytogenetic errors, and primordial follicles have time to repair sublethal damage to organelles and other structures during their prolonged growth phase. However, this technology itself raises a challenging question of how to facilitate the growth of immature oocytes in the early follicles from frozen ovarian tissue.

The maturation of ovarian follicles is a complex process involving an optimal hormonal milieu and some known and unknown growth factors. There is accumulating evidence that, in addition to oestradiol and follicle stimulating hormone (FSH), polypeptide growth factors, including insulin-like growth factors (IGF) and transforming growth factor-β (TGFβ), may play roles in the regulation of the development and function of follicles in animals and humans (Adashi et al., 1985; Giudice, 1992; Gougeon, 1996). Many studies have revealed that insulin-like growth factors I and II (IGF-I and IGF-II) are involved in the local control of ovarian function (Adashi et al., 1985; Giudice, 1992; Gougeon, 1996). IGF-I promotes the differentiation and proliferation of rat granulosa cells, synergizes with pituitary gonadotrophins and augments the effect of FSH on the synthesis of oestradiol, progesterone and inhibit and on the induction of luteinizing hormone (LH) receptors (Adashi et al., 1985). IGF-I, in combination with gonadotrophins, enhances the in-vitro maturation of pig and bovine oocytes and the subsequent developmental competence of the embryos (Harper and Brackett, 1992; Xia et al., 1994). The availability of biologically active IGF in tissue fluid is regulated by specific IGF binding proteins (IGFBP), of which there are at least six structurally different forms (IGFBP-1 to IGFBP-6). TGFβ may regulate IGF by controlling the secretion of IGFBP. The IGF-I receptor comprises a heterotetrameric structure of two α and two β subunits, which are involved in ligand binding and tyrosine kinase activity respectively. With
receptor binding (Adashi et al., 1985; Spicer et al., 1994), immunohistochemical staining (Balboni et al., 1987; Samoto et al., 1993) and quantitative flow cytometric analysis (De Neubourg et al., 1998), the presence of IGF-I receptors has been proved in granulosa cells of animals and human.

TGFβ is a family of dimeric 25 kDa proteins that are...
In the present study, the expression of IGF-IR, TGF-β2 and TGF-β type I receptor in follicles of human ovarian tissue was investigated in different cellular components of follicles. The choice of TGF-β type I receptor was based on the knowledge that TGF-β has been shown to modulate many biological events in rat and domestic animal ovaries (Knecht et al., 1989). TGF-β1 inhibits ovarian androgen production (Hernandez et al., 1990), presumably by directly inhibiting ovarian 17α-hydroxylase in theca–interstitial cells (Fournet et al., 1996). In the human ovary, TGF-β1 stimulates FSH-induced aromatase activity in cultured granulosa–luteal cells (Rabino civici et al., 1992). The effect of TGF-β is mediated by heteromeric types I and II serine and threonine kinase TGF-β receptors, TβR-I and TβR-II (Hu et al., 1998). It has been suggested that a third receptor may modulate the binding of ligand to the other receptors (Hu et al., 1998).

In order to mature human oocytes in early follicles from cryopreserved ovarian tissues in vitro for the IVF programme in our laboratory, the aim of this study was to understand the effects of various hormones and growth factors and the role of their receptors in the regulation of follicular development. In the present study, the expression of IGF-IR, TβR-I, and TβR-II was investigated in different cellular components of follicles of human ovarian tissues before and after cryopreservation.

| Table I. Immunohistochemical staining for insulin-like growth factor (IGF) type I receptor in follicles of human ovarian tissue |
|-----------------------------|------------------|------------------|------------------|------------------|
| Follicle       | Oocyte | Granulosa cell | Theca cell | Stroma cell |
| Primordial     | +++    | +               | –            | +++           |
| Pre-antral     | +++    | ++              | +            | +             |
| Antral         | + + +  | ++              | +++          | +             |

Staining grades: (−) no immunostaining; (++) weak staining; (++) moderate staining; (+++) strong staining.

| Table II. Immunohistochemical staining for transforming growth factor-β type I receptor in follicles of human ovarian tissue |
|-----------------------------|------------------|------------------|------------------|------------------|
| Follicle       | Oocyte | Granulosa cell | Theca cell | Stroma cell |
| Primordial     | +++    | +               | –            | +++           |
| Pre-antral     | +++    | ++              | +            | +             |
| Antral         | + + +  | ++              | +++          | +             |

Staining grades: (−) no immunostaining; (++) weak staining; (++) moderate staining; (+++) strong staining.

identified in mammals as five structurally closely related isotypes, TGFβ1 to TGFβ5, which have virtually identical biological activities (Hu et al., 1998). In the human ovary, TGFβ1 is present in most ovarian cell types, while intense immunostaining for TGFβ2 has only been detected in thecal cells (Chegini and Flanders, 1992). The effects of TGF-β on the growth and differentiation of human ovarian cells are not well known. TGF-β have been shown to modulate many biological events in rat and domestic animal ovaries (Knecht et al., 1989). TGFβ1 inhibits ovarian androgen production (Hernandez et al., 1990), presumably by directly inhibiting ovarian 17α-hydroxylase in theca–interstitial cells (Fournet et al., 1996). In the human ovary, TGFβ1 stimulates FSH-induced aromatase activity in cultured granulosa–luteal cells (Rabino civici et al., 1992). The effect of TGF-β is mediated by heteromeric types I and II serine and threonine kinase TGFβ receptors, TβR-I and TβR-II (Hu et al., 1998). It has been suggested that a third receptor may modulate the binding of ligand to the other receptors (Hu et al., 1998).

In order to mature human oocytes in early follicles from cryopreserved ovarian tissues in vitro for the IVF programme in our laboratory, the aim of this study was to understand the effects of various hormones and growth factors and the role of their receptors in the regulation of follicular development. In the present study, the expression of IGF-IR, TβR-I, and TβR-II was investigated in different cellular components of follicles of human ovarian tissues before and after cryopreservation.

Materials and methods

Ovarian tissue

Ovarian tissues were collected from 14 women (aged 21–37 years) with regular menstrual cycles who underwent gynaecological evaluation. All the patients had received diagnostic laparoscopy and biopsy of ovarian tissues. None of the patients was taking medication. Approval of this study was obtained from the Ethics Committee of the Catholic University of Louvain. Ovarian tissues were transported from the operating room to the laboratory in ice-cold Leibovitz L-15 medium supplemented with glutamax (Gibco, Paisley, Scotland). Each ovarian tissue sample was dissected into small pieces (1×1×1 mm). Some pieces were fixed in Bouin’s solution for immunohistochemical study, and assigned to the fresh ovarian tissue group. The other pieces were processed using a programmable freezer and stored in liquid nitrogen as the frozen ovarian tissue group. After various periods of time, frozen tissues were thawed and fixed in Bouin’s solution for further immunohistochemical study.

Freezing and thawing

Freezing of ovarian tissue was performed according to a modified method of Gosden et al. (1994). Cryoprotective medium, Leibovitz’s medium containing 2% human albumin (Red Cross, Brussels, Belgium) and 10% dimethylsulphoxide (DMSO) (Sigma, St Louis, USA), was dispensed into pre-cooled cryogenic vials (Simport, Quebec, Canada). The tissue fragments were suspended in the cryoprotective medium on ice for 30 min before cooling was initiated. The cryovials were loaded in the straws of a programmable freezer (Kryo 10 Series III, Planer, Sunbury on Thames, UK) and frozen using the following programmes: (i) cooled from 0°C to −8°C at −2°C/min; (ii) seeded manually by touching the cryotubes with forceps pre-chilled in liquid nitrogen; (iii) cooled to −40°C at −0.3°C/min; (iv) cooled to −150°C at −30°C/min; (v) transferred to liquid nitrogen (−196°C) immediately for storage.

For thawing, the cryovials were warmed at room temperature for 2 min, and immersed in a water bath at 37°C for another 2 min. The tissues were immediately transferred from the cryovials to tissue Petri dishes (Becton Dickinson, Meylan Cedex, France) added with fresh Leibovitz’s medium, and subsequently washed three times with the same medium to remove cryoprotectant before further processing.

Immunohistochemical staining

Fixed fresh and frozen fragments of ovarian tissue were dehydrated with ethanol, cleaned with toluene and embedded in paraffin. A series of continuous sections 6 μm thick was prepared from ovarian tissue fragments.

Immunohistochemical staining was performed using avidin–biotin immunoperoxidase system. The sections were deparaffinized with toluene, rehydrated in ethanol and quenched in 3% hydrogen peroxide for 30 min at 37°C. After washing in water and Tris-buffered solution (TBS) (Dako, Glostrup, Denmark), normal goat serum (Dako) was applied for 1 h at 37°C. The antibody against human IGF type I receptor (200 μg/ml) and the antibodies against human TGFβ type I (200 μg/ml) and TGFβ type II receptors (Dako) were all purchased from San Cruz Biotechnology (Santa Cruz, CA, USA) and applied as the primary antibodies. These antibodies were prepared in antibody diluent (Dako) at dilutions of 1:400 for anti-IGF type I receptor antibody, 1:300 for anti-TGFβ type I receptor antibody and 1:10 for anti-TGFβ type 2 receptor antibody, respectively. The sections were incubated with the appropriate antibodies in a sealed humidified chamber overnight at 4°C. After washing with TBS, the specimens were incubated with biotinylated anti-rabbit secondary antibody (Boehringer Mannheim, Brussels, Belgium) at a dilution of 1:250 for 30 min at room temperature. Streptomyacin avidin–peroxidase conjugate (Boehringer Mannheim) was added and incubated for 30 min at room temperature. Chromogenic reaction was developed by incubation with a freshly prepared solution of 3,3′-diaminobenzidine (DAB) (Dako). The sections were counterstained
Figure 2. Immunohistochemical staining for transforming growth factor-β type I receptor (TβR-I) in follicles of human ovarian tissues before and after cryopreservation (bar = 35 µm). (A) No staining in primary follicle in the control section of fresh ovarian tissue; (B) staining for TβR-I in primary follicle in fresh ovarian tissue; (C) staining for TβR-I in pre-antral follicle (solid arrowhead) and primordial follicle (open arrowhead) in fresh ovarian tissue; (D) staining for TβR-I in theca cells in fresh ovarian tissue; (E) staining for TβR-I in primordial follicle in frozen ovarian tissue; (F) staining for TβR-I in antral follicle in frozen ovarian tissue.

with Mayer’s haematoxylin (Merck, Darmstadt, Germany). Serial sections were used to compare the staining patterns with different primary antibodies. Tissue sections of all 14 women were subjected to immunohistochemical staining for the three receptors. (No significant differences in staining patterns between different women were observed.) Control procedures were undertaken simultaneously to assure the specificity of immunoreaction. Two negative controls were carried
out by replacing the primary antibodies with (1) phosphate-buffered solution (PBS) and (2) rabbit immunoglobulin G (IgG) (Sigma) at the same dilutions as for the specific primary antibodies.

All the slides were examined by the same observer who was blind to the tissue sections between fresh and frozen groups. The intensity of immunohistochemical staining was graded semiquantitatively as follows: (−) no immunostaining; (+) weak staining; (+++) strong staining.

Results

Expression of IGF type I receptor in follicles of human ovarian tissue before and after cryopreservation

Expression of IGF type I receptor (IGF-IR) was studied immunohistochemically in a series of continuous sections of human ovarian tissue before and after cryopreservation.

Immunoreactivity of IGF-IR was detected in follicles of fresh ovarian tissue at various developmental stages (Figure 1B–D) (Table I). Staining for IGF-IR was present in the oocytes of primordial follicles, the earliest stage of follicular development. Immunostaining for IGF-IR was also constantly observed in the oocytes of pre-antral and antral follicles. IGF-IR was expressed weakly in pre-granulosa cells of primordial follicles. Staining for IGF-IR was intensified in granulosa cells from pre-antral to antral follicles. IGF-IR staining was much stronger in thecal cells than in oocytes and granulosa cells. Stromal cells also showed IGF-IR immunoreactivity in ovarian tissue at different follicular stages. In the control sections, replacing the primary antibody with rabbit IgG resulted in a complete absence of staining in ovarian tissue (Figure 1A), indicating the specificity of immunostaining for IGF-IR.

To study the possible influence of freezing on IGF-IR immunoreactivity, immunostaining for IGF-IR was investigated in frozen ovarian tissue (Figure 1E and F). No significant difference in the staining pattern for IGF-IR was observed in frozen ovarian tissue, compared to fresh ovarian tissue. The IGF-IR staining intensity was similar in the oocytes and granulosa cells of primordial, pre-antral and antral, in both frozen and fresh ovarian tissues. The strongest staining for IGF-IR was observed in thecal cells of antral follicles. Positive staining for IGF-IR was also found in stromal cells.

Expression of TGFβ type II receptor in follicles of human ovarian tissue before and after cryopreservation

Expression of TGFβ type II receptor was observed in follicles of fresh ovarian tissue during follicular development (Table II). Staining for TβR-I was found in the oocytes of primordial, pre-antral and antral follicles (Figure 2B–D). Immunostaining for TβR-I was also observed in pre-granulosa cells of primordial follicles, as well as in granulosa cells of pre-antral and antral follicles. The strongest immunoreactivity of TβR-I was detected in thecal cells. In addition, staining for TβR-I was observed in stromal cells at different stages.

In frozen ovarian tissue, the expression of TβR-I was similar to that observed in fresh ovarian tissue (Figure 2E and F). Immunostaining for TβR-I was present in oocytes from primordial to antral follicles. Immunostaining was also found in granulosa cells from primordial to antral follicles. The strongest staining for TβR-I was found in thecal cells of antral follicles. Positive staining for TβR-I was also observed in stromal cells.

Expression of TGFβ type I receptor in follicles of human ovarian tissue before and after cryopreservation

TGFβ type II receptor was expressed in follicles of fresh ovarian tissue, but at a much weaker staining intensity, compared to TβR-I (Table III) (Figure 3A–C). Faint immunostaining for TβR-II was only found in the oocytes of primordial and primary follicles, and disappeared in large pre-antral and antral follicles. Weak staining for TβR-II was also present in thecal cells. No significant immunostaining was detected in granulosa cells of pre-antral and antral follicles.

Immunoreactivity of TβR-II was also detected in follicles of frozen ovarian tissue (Figure 3E and F). Immunostaining for TβR-II in frozen tissue was similar to that in fresh tissue. Immunostaining for TβR-II was found in the oocytes of primordial and primary follicles, but not in large pre-antral and antral follicles. Weak TβR-II staining was also encountered in thecal cells of antral follicles. No significant staining was observed in granulosa cells of pre-antral and antral follicles.

Discussion

The presence of IGF type I receptor has been studied previously in the ovaries of various animal species and humans (Giudice, 1992). IGF type I receptor was identified by binding assays in rat granulosa cells and immunohistochemical staining in human oocyte, granulosa and theca of antral, pre-ovulatory and atretic follicles (Davoren et al., 1986; Balboni et al., 1987; Gates et al., 1987; Samoto et al., 1993). Affinity cross-linking study revealed the binding of IGF-I to a protein of Mr ~130 000 in granulosa membrane preparation, consistent with the α-subunit of the type I receptor (Gates et al., 1987). IGF type I and type II receptor mRNA were detected in granulosa-luteal cells of the human ovary (El Roeiy et al., 1993). Recently, De Neubourg et al. (1998) quantified with flow cytometry the IGF type I receptor on granulosa cells in follicular fluid from dominant follicles.
However, study of IGF receptor expression in early follicles of the human ovary is still limited.

In the present study, it has been demonstrated, for the first time, that IGF type I receptor is present in the very early follicles of human ovarian tissue. Immunostaining for IGF type I receptor was observed constantly in the oocytes and granulosa cells of primordial, pre-antral and antral follicles. IGF type I receptor was also found in thecal cells and stromal
cells. These results corroborate previous findings (Giudice, 1992; El-Roeiy et al., 1993; Spicer et al., 1994), suggesting that IGF type I receptor may be involved in the regulation of the growth of immature oocytes starting from primordial follicular stages, in human ovarian tissue.

The role of IGF-I and the interaction with its receptor in the initiation and regulation of oocyte growth in primordial follicles is unclear. It has been demonstrated that IGF-I stimulated nuclear maturation of cumulus-enclosed oocytes in animals and humans (Gomez et al., 1993; Yoshimura et al., 1996; Pawshe et al., 1998), and improved early embryonic development (Pawshe et al., 1998). The action of IGF-I is mediated by the IGF type I receptor because anti-IGF-I antibody blocked the stimulatory effect of IGF-I on oocyte maturation (Yoshimura et al., 1996). The observation in this study of IGF type I receptor in oocytes of primordial and primary follicles supports the previous findings, implying that IGF-I may exert an effect on oocyte maturation in early follicular stages by binding to the cell surface receptors when cumulus–oocyte complexes are not yet formed.

IGF influence a variety of biosynthetic processes and meiotic activity in ovarian granulosa and thecal cells (Giudice, 1992). IGF-I alone, or in synergy with FSH and LH, stimulated the proliferation and synthesis of steroid hormones in human granulosa–luteal cells (Olsson et al., 1990; Erickson et al., 1991). IGF was regarded as a co-gonadotrophin of LH in stimulating steroidogenesis in theca–interstitial cells (Duleba et al., 1999). These findings argue strongly that granulosa and thecal cells are the target of IGF-I, through which IGF-I regulates the maturation of oocytes in human follicles. However, this regulatory mechanism cannot entirely explain the action of IGF-I in pre-granulosa cells of primordial follicles. In this study, the presence of IGF type I receptor was identified in a single layer of cells around primordial follicles that were not yet mature enough for secretory function. Therefore, IGF-I is more likely to stimulate the proliferation or maturation, but not to enhance steroidogenesis of the pre-granulosa in the early follicles.

The granulosa cell is proposed as the main site of IGF-I production in human follicles (Giudice et al., 1992). IGF-I is detectable in follicular fluid from IVF patients (Oosterhuis et al., 1998). However, immunohistochemical studies showed that there was no follicular site other than the theca for IGF-I production or its mRNA expression (Voutilainen et al., 1996; De Neubourg et al., 1998). Unlike IGF-II, the concentration of IGF-I was lower in ovarian venous blood samples than in peripheral venous samples (Jesionowska et al., 1990). These reports suggest that IGF-I in follicular fluid may derive from the circulation, and thus extraovarian IGF-I may serve as an endocrine factor in regulating folliculogenesis (Jesionowska et al., 1990). In this study, IGF type I receptor was found to be present in the oocyte and pre-granulosa cells in primordial follicles. It seems that oocytes and pre-granulosa cells are targeted by circulating IGF-I, rather than the IGF-I that is produced locally within the early follicles, through autocrine and paracrine mechanisms.

The growth-promoting and differentiating actions of IGF-I may be mediated through the IGF type I receptor, rather than

the IGF type II receptor (Adashi et al., 1990). Abundant IGF-II mRNA was found exclusively in granulosa cells of antral and dominant follicles (El-Roeiy et al., 1993). The possibility that the IGF-II produced by the granulosa and other cells binds to the IGF type I receptor in regulating oocyte maturation in the early follicles through a paracrine mechanism cannot be excluded.

Like IGF, TGFβ are important intra-ovarian regulators of folliculogenesis. TGFβ3 was localized immunocytochemically in ovarian granulosa and theca–interstitial cells (Mulheron et al., 1992; Gangrade et al., 1993; May et al., 1996). The dominant form present in granulosa cells is TGFβ1 (Mulheron et al., 1992; Teerds and Dorrington, 1992; Gangrade et al., 1993). Both theca–interstitial and granulosa cells secreted TGFβ in cultures (Mulheron et al., 1992). An increase in amounts of TGFβ was recently observed in ovarian follicular fluid in women following ovarian stimulation for IVF (Fried et al., 1998). TGFβ differentially regulated DNA synthesis and the proliferation of granulosa cells (May et al., 1994) and induced apoptosis in rat theca–interstitial cells (Foghi et al., 1997). TGFβ enhanced basal and gonadotrophin-stimulated steroidogenesis in granulosa and thecal cells (Dodson and Schomberg, 1987; Magoffin et al., 1989; Fournet et al., 1996). TGFβ also stimulated meiotic maturation of rat oocytes (Feng et al., 1988), although both inhibin and TGFβ suppressed LH-induced oocyte maturation in pre-ovulatory follicles (Tsafiri et al., 1989).

The action of TGFβ is mediated through binding to its receptors on the cell surface (Hu et al., 1998). TGFβ receptors are present in ovarian tissues of hamsters (Roy and Kole, 1995), bovines (Roelen et al., 1998) and humans (Roy and Kole, 1998). Roy and Kole (1995) first identified the presence of the TGFβ type II receptor protein, in both membrane-associated and cytosolic forms, in hamster granulosa cells of pre-antral and antral follicles and interstitial and luteal cells. The induction of TGFβ type II receptor was regulated by gonadotrophins and steroid hormones including FSH, LH, 17β-oestradiol, progesterone and testosterone. TGFβ type I and II receptors were also found to be present in granulosa, thecal and interstitial cells of human pre-antral follicles (Roy and Kole, 1998). Recently, Roelen et al. (1998) reported the expression of mRNA for TGFβ type I and II receptors in oocytes from the bovine ovary. In the present study, the results support the previous findings by Roy and Kole (1998), indicating that TGFβ receptor is present in the follicles of the human ovary. It was demonstrated that the TGFβ type I receptor was constantly expressed in the oocytes of primordial, pre-antral and antral follicles in human ovarian tissue. The TGFβ type II receptor was also found in the oocytes of primordial and primary follicles. These observations suggest that TGFβ may participate in the modulation of oocyte maturation through binding to both TGFβ type I and type II receptors in early follicles of human ovarian tissue. In this study, it was further observed that TGFβ type I receptor was also expressed with intense staining in granulosa and thecal cells of pre-antral and antral follicles. This observation corroborates the previous reports on the roles of TGFβ and related peptides in regulating the differentiation and secretory
function of ovarian granulosa and thecal cells (Knecht et al.,
1989; Erämaa and Ritvos, 1996).

In the present study, it was observed that the expression of TGFβ type II receptor was distinct from the type I receptor with respect to cellular distribution in ovarian tissue. TGFβ type II receptor stained only in oocytes of primordial and primary follicles, faintly in thecal cells, and no significant staining in oocytes and granulosa cells in pre-antral and antral follicles. These results are not consistent with the report by Roy and Kole (1998) on the expression of TGFβ type II receptor in granulosa, thecal and interstitial cells in the ovary. The reason for the discrepancy is unknown.

The significance of the concomitant presence of TGFβ receptors and IGF type I receptor in early follicles is yet to be illuminated. Several studies have implied a relationship between TGFβ and IGFs in hormonal regulation. Both TGFβ1 and IGF-I were detectable in human ovarian follicular fluid (Fried et al., 1998). TGFβ exerted a stimulating effect on basal and epidermal growth factor-induced production of IGF-I in porcine granulosa cell cultures (Mondschein et al., 1988). TGFβ might regulate the effect of IGF by controlling the secretion of IGF binding proteins (Martin and Baxter, 1991). Following these findings, the results of the current study further suggest that both IGF-I and TGFβ may be involved in a sophisticated paracrine–autocrine network in the regulation of oocyte maturation and follicular growth in the ovary.

This study is one part of our research project on human ovarian tissue banking and in-vitro maturation of oocytes in early follicles. It could be worthwhile further investigating the possibility of using IGF and TGFβ to regulate the in-vitro or in-vivo growth of oocytes in early follicles from cryopreserved ovarian tissue. Therefore the possible influence of cryopreservation on the immunoreactivities of IGF-I and TGFβ receptors in follicles of frozen ovarian tissue was also studied. The results show that there was no significant difference in immunostaining for either IGF type I receptor or TGFβ type I and II receptors in follicles of frozen ovarian tissues, compared to fresh tissues from pair-controlled samples. It seems that freezing does not significantly alter the immunoreactivities of IGF type I receptor and TGFβ type I and II receptors in follicles after ovarian tissue cryopreservation.

In conclusion, the results demonstrate that receptors of IGF-I and TGFβ were expressed in follicles of human ovarian tissue. These receptors may mediate the effects of IGF-I and TGFβ in the regulation of follicular growth by a paracrine or autocrine mechanism. Further studies are required to clarify the regulatory effects of these growth factors on the in-vitro or in-vivo maturation of oocytes in early follicles from cryopreserved human ovarian tissue in the future.

Acknowledgements
The authors would like to thank D.Toussaint and F.Casanas-Roux for their excellent technical assistance in cryopreservation and morphological study respectively. We also express our thanks to Dr E.Marbaix (Department of Pathological Anatomy) for the help in morphological study and the comment on the manuscript. We also appreciate P.Camby (Department of Pathological Anatomy) for the help with immunohistochemical study and W.J.Hudders (Department of Histology) for photography. This study was supported by grants from F.N.R.S.3.4568.97 and Televie 7.4530.98, Belgium.

References
localization, mechanisms(s) and site(s) of action. Endocrinology, 127, 2804–2810.


Received on June 9, 1999; accepted on November 1, 1999