Immunolocalization of Fas and Fas ligand in the ovaries of women with polycystic ovary syndrome: relationship to apoptosis*

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Both Fas (APO-1, CD95), an apoptosis-inducing receptor, and its ligand, Fas ligand (FasL, CD95L), have been localized to the ovary. Granulosa cell apoptosis occurs in antral follicular atresia. In polycystic ovary syndrome (PCOS), antral follicles accumulate with some atretic features. The ovarian expression of Fas and FasL was examined in PCOS by immunohistochemistry and correlated with immunodetection of apoptotic cells. Fas immunostaining was present in pre-antral follicle oocytes, some primary and secondary pre-antral follicle granulosa cells, and both granulosa and theca of antral follicles. Thecal staining persisted with advancing atresia, while granulosa staining declined. In antral follicles, abundant Fas-positive cells co-localized with scattered nuclei immunopositive for apoptosis. Ovarian vascular myocytes were strongly Fas-immunopositive. FasL immunostaining was present in pre-antral follicle oocytes and variably in granulosa. In antral follicles, granulosa and thecal FasL staining increased with advancing atresia. Normal control ovaries showed follicular Fas and FasL staining patterns similar to those in PCOS, but vascular staining was less prominent. In one healthy follicle, Fas immunostaining was seen in the oocyte and weakly in mural granulosa and theca interna. The results suggest that in PCOS, an alteration in Fas-mediated apoptosis, does not cause abnormal folliculogenesis, but may promote ovarian vascular remodelling. Key words: apoptosis/Fas/Fas ligand/human ovary/polycystic ovary syndrome

Introduction

Fas (APO-1, CD95) is a member of the tumour necrosis factor (TNF-α) receptor superfamily which, when ligated, can signal a cell to undergo apoptosis, or programmed cell death. Fas can be activated through trimerization by Fas ligand (FasL, CD95L), a transmembrane protein in the TNF-α superfamily, or by some anti-Fas antibodies. Fas activation leads to proteolytic activation of members of a family of cysteine proteases called caspases, which then leads to apoptosis. Although Fas was first characterized in the immune system, it is expressed in diverse tissues (Leithäuser et al., 1993; Nagata and Golstein, 1995). FasL is expressed both on lymphocytes and on epithelial cells in sites of immunoprivilege; in the latter, it may be viewed as a defence against Fas-expressing activated lymphocytes (Griffith et al., 1995; Runic et al., 1996).

In the ovary, apoptosis occurs during both antral follicular atresia and luteal regression (Hsueh et al., 1994; Shikone et al., 1996; Yuan and Giudice, 1997). These normal cyclic processes have been attributed to decreasing gonadotrophic support (Chun et al., 1996), but the molecular basis of apoptosis initiation in these tissues is not understood.

Several studies have suggested a possible role of Fas in ovarian apoptosis. In rodents, Fas is expressed on granulosa cells of early atretic follicles and luteal cells of regressing corpora lutea, but not on granulosa cells of healthy follicles or oocytes (Hakuno et al., 1996; Kim et al., 1998; Roughton et al., 1999). FasL expression was found only on oocytes in the rat in one study (Hakuno et al., 1996), but on granulosa cells of mouse and rat antral follicles in two others (Mori et al., 1997; Kim et al., 1998). Exposure of cultured granulosa cells to zona-denuded oocytes induced apoptosis, which could be blocked by Fas antiserum (Hakuno et al., 1996). In humans, luteinizing granulosa cells express Fas and undergo apoptosis when treated with agonist anti-Fas antibody (Quirk et al., 1995; Cataldo and Jaffe, 1996). In the normal human ovary, Fas has been immunolocalized to both granulosa and theca cells of atretic, but not healthy, antral follicles (Kondo et al., 1996). These findings, taken together, suggest that Fas/FasL may mediate apoptosis in antral follicles.

In women, polycystic ovary syndrome (PCOS) is characterized by ovarian hyperandrogenism and anovulation resulting from a disorder of follicular maturation of uncertain aetiology. The antral follicles that accumulate in the PCOS ovary, like atretic antral follicles in ovulatory women, contain androgen-dominant fluid and fewer granulosa cells than healthy follicles (McNatty et al., 1979; Erickson and Yen, 1984). Antral follicles in the PCOS ovary may not all be truly atretic, however, since their granulosa cells can increase aromatase activity in response to exogenous FSH both in vitro (Erickson et al., 1992; Andreani et al., 1994; Almabhobi et al., 1996) and in vivo (Meirow et al., 1993), and their oocytes can be matured in vitro (Trounson et al., 1994).

In the present study, we examined the expression of Fas and FasL by immunohistochemistry in the ovaries of women with PCOS and related these observations to apoptosis, assessed by immunodetection of fragmented DNA. Additionally, the expression patterns of Fas and FasL in the PCOS ovary were compared with those in normal cycling ovaries. The observed patterns of immunolocalization of Fas and FasL were similar in the follicles of PCOS and cycling control ovaries, and therefore do not support a unique role for these molecules in the disordered folliculogenesis of PCOS.

**Materials and methods**

**Sources of tissue**
Archival formalin-fixed, paraffin-embedded blocks of human ovarian tissue were used under an Institutional Review Board exemption granted by the University of California, San Francisco (UCSF). Ovarian tissue was studied from five women with PCOS and three normally cycling women. The PCOS subjects ranged in age from 34 to 41 years, had hirsutism plus oligomenorrhea or amenorrhea, or had histological evidence of PCOS. All had undergone hysterectomy and bilateral oophorectomy for clinical indications. The normal subjects were aged 36–45 years, had undergone oophorectomy for benign disease, and had histologically normal ovaries. Two of these subjects also had undergone hysterectomy and showed proliferative-phase endometrium; the third had undergone oophorectomy on cycle day 21. An archival block of human term placenta was used as a positive control for FasL staining (Runic et al., 1996).

**Immunostaining for Fas and FasL**
Tissue blocks were cut on a microtome at 5 μm thickness and serial sections mounted on acid-washed, silanized slides. After deparaffinization and rehydration, tissue sections were quenched with 0.1% hydrogen peroxide, equilibrated in 0.05 mol/l Tris-Cl–0.15 mol/l NaCl, pH 7.3 (TBS), and blocked with 2% normal horse serum (NHS), prior to incubation overnight at 4°C with a 1:100 dilution of mouse monoclonal anti-human Fas IgG (UB2; Kamiya Biomedical Corp., Seattle, WA, USA). After washing with TBS and blocking with 2% NHS, bound antibody was detected by the avidin-biotin-peroxidase method, using reagents of the Vectastain ABC kit (Vector Labs, Burlingame, CA, USA). Biotinylated anti-mouse IgG was used at 1:200. For FasL immunostaining, sections were blocked with 3% normal goat serum (NGS) in TBS and incubated overnight with a 1:100 dilution of rabbit polyclonal anti-rat FasL antisemur (N-20; Santa Cruz Biototechnology, Santa Cruz, CA, USA). Sections were then blocked with 3% NGS and treated with 1:100 goat anti-rabbit antisemur (ICM Biomedical, Costa Mesa, CA, USA) for 30 min at 22°C, followed by incubation in 1:100 rabbit peroxidase-anti-peroxidase reagent (ICN). For both antigens, bound peroxidase was then reacted with diaminobenzidine (DAB) substrate and H2O2, and the tissue lightly counterstained with haematoyxlin, dehydrated, and covered with a coverslip. As negative controls, adjacent sections were reacted in parallel with substitution of buffer for the primary antibody.

**Detection of apoptosis**
Sections adjacent to those used for Fas immunostaining were analysed for apoptosis using the Apoptag kit (Oncor, Gaithersburg, MD, USA) to detect oligonucleosomal DNA fragmentation. The manufacturer’s protocol was followed, except that TBS was substituted for phosphate-buffered saline. After deparaffinization and treatment with proteinase K (BRL, Gaithersburg, MD, USA; 20 μg/ml), tissue sections were incubated with terminal transferase and digoxigenin-dUTP to label free ends of chromosomal DNA. Digoxigenin was then detected immunohistochemically with horseradish peroxidase-coupled antidigoxigenin antibody and DAB. After counterstaining with haematoyxlin, apoptotic nuclei appeared an intense brown, while non-apoptotic nuclei took up only the blue counterstain. Negative controls were reacted in parallel with the omission of terminal transferase.

**Imaging**
Photomicrographs were taken on a Leica DMRB microscope with a Canon A2 (Elan II) camera and Ektachrome ISO 64 tungsten film (EPY64; Kodak, Rochester, NY, USA). Transparencies were digitized with a Polaroid SprintScan 35 scanner and Macintosh computer into Adobe Photoshop (Adobe Systems, San Jose, CA, USA). Image brightness, contrast, and sharpness were adjusted to optimize image quality, and colour was adjusted to improve consistency among images. Scale bars are shown on representative images of each magnification in each figure.

**Classification of follicles**
An apparently healthy antral follicle contained numerous granulosa cell layers with evident mitoses and little if any pyknosis, and a well-condensed theca interna; antral borders were smooth and regular. Antral follicles in PCOS and the atretic antral follicles in cycling controls were classified histologically according to the scheme of Gougeon for atretic follicles (Gougeon, 1993). Stage A follicles contained at least five layers of granulosa cells, with a regular shape and only scattered pyknotic nuclei. Stage B follicles had fewer granulosa cell layers and more prominent pyknosis; thecal architecture was preserved. In PCOS, hypertrophy and luteinization were sometimes seen. Stage C follicles showed scant granulosa cells, or fibrosis or hyalinization of this layer. In the PCOS ovaries, the theca appeared hypertrophied or luteinized in some follicles, but degenerating in others.

**Results**

**Normal cycling ovary**

**Histology**
Evaluation of follicles in all stages of development indicated that the vast majority of antral follicles were morphologically atretic. Only one antral follicle, ~1 mm in diameter, could be identified as healthy because of its abundant granulosa cell mitoses and well-preserved Graafian architecture.

**Immunolocalization of Fas**
Oocytes of pre-antral follicles were consistently immunopositive, as were granulosa cells of primary and later pre-antral stages; in primordial follicles, nearly all pre-granulosa cells were negative (Figure 1A). In the healthy antral follicle, staining for Fas occurred in the oocyte and diffusely in the theca interna. Mural granulosa cells at the antral surface were moderately immunopositive, while cumulus granulosa cells were negative near the oocyte but showed slight immunostaining in the mitotic region closer to the basement membrane (Figure 2). In stage A atretic antral follicles, Fas immunostaining was concentrated in juxtaluminal granulosa cells and a zone of theca interna cells close but not immediately adjacent to the basement membrane (Figure 3, panel A1). In stages B and C, staining persisted both in the juxtaluminal granulosa cells and in the theca interna (Figure 3, panels B1 and C1).
Figure 1. Immunohistochemical staining for Fas and FasL in pre-antral follicles. Representative primordial (Pmd), primary, and secondary follicles in ovarian sections from women with normal cycles (A, B) and women with PCOS (C, D) are shown after immunostaining with a specific antibody against Fas (A, C) or FasL (B, D), or with substitution of buffer for the primary antibody as a control (Con). All sections were lightly counterstained with haematoxylin. Scale bars, shown for control sections, represent 25 µm (Pmd, Primary); or 50 µm (Secondary). Matched pairs of specifically immunostained and control sections are shown at the same scale.

Figure 2. Immunohistochemical staining for Fas in a healthy antral follicle. Sections of an apparently healthy antral follicle, 1 mm in diameter, from a normally cycling subject have been immunostained with a specific Fas antibody (Fas) or with the buffer vehicle alone as a control (Con). All sections were lightly counterstained with haematoxylin. Fas immunostaining appears in the oocyte (o), in basal cumulus (c) (shown in Cumulus panels), and in granulosa (g) and theca (t) of the antral wall (shown in Mural panels). There are several mitotic figures in the basal cumulus (inset on Cumulus panel). Boxes in the Overview Con panel indicate the approximate locations of the Cumulus and Mural panels. Scale bars represent 200 µm (Overview); 50 µm (Cumulus); or 25 µm (Mural).

One haemorrhagic corpus luteum showed focal staining of nests of granulosa-lutein cells; theca-lutein cells were negative (data not shown). These results are summarized in Table I. The surrounding stroma was immunonegative at all follicular stages. No staining was seen in adjacent control sections incubated without the primary antibody (Figures 1 and 2; not
Figure 3. Immunohistochemical staining for Fas and FasL in atretic antral follicles. Representative antral follicles in stages A, B, and C of atresia in ovarian sections from women with normal cycles (rows 1–2) and women with PCOS (rows 3–5) have been immunostained with a specific antibody against Fas (rows 1, 3), against FasL (rows 2, 5), or after incubation with Apoptag kit reagents (Apo, row 4). Control sections reacted without primary antibody (for Fas and FasL) or without terminal transferase (for Apoptag staining) consistently showed no specific staining (not shown). All sections were lightly counterstained with haematoxylin. Labels show granulosa (g), theca (t), and stroma (s). Scale bars represent 25 µm (rows 1–4); 50 µm (panels B5, C5); or 100 µm (panel A5).

Immunolocalization of Fas ligand

Oocytes in both primordial and primary follicles showed positive staining for FasL. Pre-granulosa cells in primordial follicles did not stain, while granulosa cells in primary follicles showed faint and inconsistent immunostaining (Figure 1B). Stage A and B atretic antral follicles showed staining of juxtaluminal granulosa cells and thecal cells close but not immediately adjacent to the basement membrane, similar to the pattern of Fas immunostaining (Figure 3, panels A2 and B2). FasL immunostaining in stage C atretic follicles was found in the flattened remnants of granulosa cells and in scattered thecal cells (Figure 3, panel C2). The results are summarized in Table I. Faint FasL immunostaining was seen in the walls of small- and medium-calibre vessels (Figure 4).

PCOS ovary

Histology

Ovarian tissue sections from women with PCOS revealed follicles at all stages of development from primordial through antral. Numerous antral follicles were encountered, many with...
irregular shape. All of these appeared atretic; no apparently healthy antral follicles were observed. Thecal hyperplasia was evident in many, but not all, PCOS antral follicles, especially in stage C of atresia.

**Immunolocalization of Fas**

Fas immunostaining in the ovaries of women with PCOS was similar to that in normally cycling subjects (Table I). In PCOS, Fas immunostaining of oocytes was seen in all primordial, primary, and secondary pre-antral follicles. Pre-granulosa cells in primordial follicles were predominantly immunonegative, with only a few exhibiting weak staining. Granulosa cells were generally Fas immunopositive in primary follicles and consistently positive in secondary follicles (Figure 1C). Stage A atretic antral follicles showed specific cytoplasmic staining in both theca and granulosa cells, with the latter more intensely stained adjacent to the follicular lumen in some sections (Figure 3, panel A3). In stage B atretic follicles, Fas immunostaining of the theca interna persisted, while staining of the granulosa was generally less intense than in stage A (Figure 3, panel B3). Stage C atretic follicles showed intense thecal staining, while the flattened remnants of granulosa cells displayed variable immunoreactivity (Figure 3, panel C3). The surrounding stroma was immunonegative at all stages of follicular atresia. In addition to Fas staining of follicular cells, a strong positive signal for Fas was consistently detected in vascular myocytes in the PCOS ovary, with apparent venules of medium calibre showing more intense staining than small-calibre arteries; endothelial cells were negative (Figure 4, panels A3, B3). This staining appeared more intense and consistent than in vessels of comparable size in the normal ovary.

**Immunolocalization of Fas ligand**

In PCOS ovaries, FasL immunostaining was seen in oocytes of primordial and primary follicles. Pre-granulosa and granulosa cells were generally immunonegative, but faint staining was observed in some granulosa cells of both primary and secondary follicles (Figure 1D). In atretic antral follicles, weak FasL immunopositivity of both granulosa and thecal cells was seen in stages A and B; staining was prominent in both layers in stage C (Figure 3, panels A5, B5, and C5). Diffuse staining of myocytes was seen in the walls of small-calibre and some medium-calibre vessels (Figure 4, panels A4 and B4). In sections of term placenta used as a positive control, syncytiotrophoblast consistently showed positive FasL staining (Runic et al., 1996) (data not shown).

**Immunodetection of apoptosis**

Nuclei of a small number of both granulosa and thecal cells in stage A atretic antral follicles were clearly labelled using the Apoptag kit (Figure 3, panel A4). In comparison, nuclear staining occurred more frequently in both granulosa and theca of stage B follicles, and was abundant in stage C thecal cell nuclei (Figure 3, panels B4 and C4). The localization of apoptotic cells in antral follicles coincided with that of Fas expression at all stages of atresia, although only a small number of nuclei stained positive after terminal-transferase labelling of DNA. Despite the widespread immunostaining for

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**Table I. Fas and FasL immunostaining in ovarian follicles in PCOS and in normally cycling women:**

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aGranulosa-lutein cells.
bTheca-lutein cells.
Figure 4. Immunohistochemical staining for Fas and FasL in ovarian blood vessels. Shown are examples of small-calibre (A) and medium-calibre (B) vessels in ovarian sections from women with normal cycles (rows 1–2) and women with PCOS (rows 3–4), stained with a specific antibody against Fas (rows 1, 3) or FasL (rows 2, 4). Control sections reacted without primary antibody consistently showed no specific staining (not shown). All sections were lightly counterstained with haematoxylin. Scale bars represent 25 µm (panels A3, A4); 50 µm (panels A1, A2, B4); or 100 µm (panels B1–3).

Discussion

Apoptosis has been demonstrated in the granulosa cells of atretic antral follicles and in regressing corpora lutea (Hsueh et al., 1994; Quirk et al., 1995; Chun et al., 1996; Shikone et al., 1996; Yuan and Giudice, 1997; Roughton et al., 1999). Fas and FasL expression have been reported in the ovary of humans and other species (Quirk et al., 1995; Cataldo and Jaffe, 1996; Hakuno et al., 1996; Kondo et al., 1996; Sakamaki et al., 1997; Kim et al., 1998; Roughton et al., 1999; Porter et al., 2000; Vickers et al., 2000). Fas activation can induce apoptosis of follicular cells in vitro, including human granulosa-lutein cells treated with interferon (IFN)-γ (Quirk et al., 1995; Cataldo and Jaffe, 1996), bovine granulosa and thecal cells treated with IFN-γ (Porter et al., 2000; Vickers et al., 2000),
and rat granulosa (Hakuno et al., 1996) and theca-interstitial cells (Foghi et al., 1998). In unpublished studies by the authors, increased apoptosis of cultured human granulosa-luteal cells obtained after exogenous gonadotrophin ovulation induction was detectable after a 6 h exposure to agonist anti-APO-1 antibody (Trauth et al., 1989) both by cellular morphological changes and by terminal transferase labelling followed by immunostaining of fragmented DNA. Apoptosis increased with up to 48 h exposure to anti-APO-1 in a fashion dependent on pretreatment with IFN-γ (data not shown). In-vivo Fas activation by administration of an agonist antibody to mice can also promote granulosa cell pyknosis, decrease ovulation rate, and decrease corpus luteum number; in the lpr strain, which expresses markedly reduced levels of Fas, increased numbers of secondary follicles and decreased numbers of large antral follicles and corpora lutea were seen, in comparison with wild-type mice (Sakamaki et al., 1997). These findings suggest that apoptosis induced by Fas activation may play a pivotal role in both follicular atresia and luteal regression.

The numerous antral follicles which accumulate in the ovary in PCOS have classical features of atretic follicles, including androgen-dominant follicular fluid and low granulosa cell numbers relative to their size (McNatty et al., 1979; Erickson and Yen, 1984). Both the granulosa cells and the oocytes of PCOS antral follicles can, however, function normally if they are removed from their follicular microenvironment (Erickson and Yen, 1984; Andreani et al., 1994; Trounson et al., 1994). These observations, as well as the normal to exaggerated stimulatory response to exogenous FSH seen in women with PCOS, lend credence to the concept that some antral follicles in the PCOS ovary are not truly atretic, but rather exhibit arrested development. The present study was performed to determine if Fas and FasL are expressed in a unique manner in the PCOS ovary, a finding that could implicate these molecules in follicular developmental failure in PCOS. Apoptotic DNA fragmentation was also examined in the PCOS ovary.

As has been reported by others (Govan and Black, 1975; McNatty et al., 1983; Erickson and Yen, 1984), most of the antral follicles in both the PCOS and normally cycling ovarian tissue sections examined appeared morphologically atretic. In addition, a hypertrophic and luteinized-appearing theca interna was frequently seen in PCOS follicles, as previously described (Govan and Black, 1975). Fas immunostaining was consistently observed in theca cells of PCOS follicles, but direct comparison with adjacent sections showed that only a small minority of theca cells demonstrated fragmented DNA. Fas-immunopositive granulosa cells were apparent in early atretic PCOS follicles, but their staining diminished or disappeared as this layer became fibrotic in late-stage atresia. Granulosa cells in stage A and B atretic follicles that stained positively for fragmented DNA also stained positively for Fas. These relationships support a role for Fas in triggering apoptosis in PCOS thecal and granulosa cells.

The pattern of Fas immunostaining found in PCOS in the present study closely resembled that found in the morphologically atretic antral follicles of cycling women both in this study and in a previous report (Kondo et al., 1996); only slight, qualitative differences were found in Fas expression between follicles of PCOS and normal ovaries. Similarly, the pattern of fragmented-DNA staining found in PCOS follicles in the present study resembles that reported in cycling women (Yuan and Giudice, 1997). Taken together, these findings are consistent with the concept, derived from both morphology and follicular fluid composition (Govan and Black, 1975; Erickson and Yen, 1984; Cataldo and Giudice, 1992), that just as in the normal ovary, in PCOS the majority of antral follicles are atretic. It has been suggested recently (Franks et al., 1998; Homburg and Amsterdam, 1998) that dysfolliculogenesis in PCOS may actually result from decreased apoptosis at an early stage of follicular growth, allowing too many follicles to develop a theca competent to synthesize androgens. In support of this concept, an increased number of developing pre-antral follicles has been identified histologically in the PCOS ovary (Hughesdon, 1982).

Immunostaining for FasL, the ligand necessary for Fas to transduce an apoptotic signal, revealed a pattern of expression in follicular cells similar to that of Fas. FasL was localized in the oocyte and weakly in the granulosa layer of pre-antral follicles, as well as in both granulosa and thecal cells of antral follicles. Our finding of FasL in somatic follicular cells is consistent with one prior report in the mouse (Mori et al., 1997) but is in contrast to a published study in the rat, in which FasL was detected only in oocytes by Western blotting (Hakuno et al., 1996). FasL expression in granulosa cells may be stimulated by gonadotrophins (Mori et al., 1997), but another study found that its expression was increased by gonadotrophin immunoneutralization (Kim et al., 1998).

Fas-mediated apoptosis could be triggered by oocyte-derived FasL in pre-antral follicles, and by either oocyte- or somatic cell-derived FasL in antral follicles. Although Fas and FasL may be present in the same or adjacent cells, and mice with Fas loss-of-function mutations accumulate medium-sized follicles or develop cystic ovaries (Sakamaki et al., 1997; Xu et al., 1998), suggesting a defect in follicular atresia, a role for these molecules in effecting follicular cell apoptosis has not been established. The mechanisms that normally prevent activation of the Fas–FasL apoptotic pathway in follicles are not known, but may involve the inhibitor of apoptosis proteins (IAP), a family of caspase inhibitors, two of which have been localized in the rat ovary and one of which, neuronal apoptosis inhibitory protein (NAIP), may function to prevent antral follicular apoptosis (Li et al., 1998; Deveraux and Reed, 1999; Matsumoto et al., 1999).

The significance of the more prominent Fas immunostaining in vascular smooth muscle in PCOS ovaries than in cycling controls is uncertain. It is noteworthy that terminal-transferase staining suggestive of apoptosis was found in myocytes of an intensely Fas-positive PCOS ovarian vessel. Fas expression in vascular smooth muscle has been previously reported (Leithäuser et al., 1993), and ovarian stroma is more highly vascular in PCOS than in normal controls, as demonstrated by colour Doppler ultrasound (Zaidi et al., 1995; Agrawal et al., 1998). Women with polycystic ovaries on ultrasound have raised serum levels of vascular endothelial growth factor (VEGF) (Agrawal et al., 1998), a potent angiogenic factor whose ovarian production is stimulated by LH/human chorionic
gonadotrophin (HCG) and insulin (Laitinen et al., 1997; Simpson et al., 1999). It is not known whether VEGF-induced angiogenesisis principally responsible for the greater stromal vascularity of the PCOS ovary, or whether alterations in Fas-mediated vascular myocyte apoptosis may also play a role in regulating vascularity, as has been recently shown in the retina (Kaplan et al., 1999).

In summary, our results demonstrate that in the ovaries of both normally cycling women and those with PCOS, Fas protein is expressed in morphologically atretic antral follicles, and that in these follicles, its staining pattern is similar in both groups of women. Our findings suggest that Fas ligation, with consequent induction of apoptosis, does not play a unique role in the failure of follicular maturation that occurs in PCOS. Our terminal-transferase staining data also indicate that apoptotic cells are prevalent, but not abundant, in antral follicles in PCOS. This finding supports the concept that at least some antral follicles in the PCOS ovary are not irreversibly committed to atresia, and therefore can be rescued by exogenous FSH.

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