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Extra- and intra-ovarian factors in polycystic ovary syndrome: impact on oocyte maturation and embryo developmental competence

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BACKGROUND: Polycystic ovary syndrome (PCOS) is a common metabolic dysfunction and heterogeneous endocrine disorder in women of reproductive age. Although patients with PCOS are typically characterized by increased numbers of oocytes retrieved during IVF, they are often of poor quality, leading to lower fertilization, cleavage and implantation rates, and a higher miscarriage rate.

METHODS: For this review, we searched the database MEDLINE (1950 to January 2010) and Google for all full texts and/or abstract articles published in English with content related to oocyte maturation and embryo developmental competence.

RESULTS: The search showed that alteration of many factors may directly or indirectly impair the competence of maturating oocytes through endocrine and local paracrine/autocrine actions, resulting in a lower pregnancy rate in patients with PCOS. The extra-ovarian factors identified included gonadotrophins, hyperandrogenemia and hyperinsulinemia, although intra-ovarian factors included members of the epidermal, fibroblast, insulin-like and neurotrophin families of growth factors, as well as the cytokines.

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CONCLUSIONS: Any abnormality in the extra- and/or intra-ovarian factors may negatively affect the granulosa cell–oocyte interaction, oocyte maturation and potential embryonic developmental competence, contributing to unsuccessful outcomes for patients with PCOS who are undergoing assisted reproduction.

Key words: polycystic ovary syndrome / oocyte / fertilization / embryo / IVF

Introduction

Polycystic ovary syndrome (PCOS) is a common metabolic dysfunction and heterogeneous endocrine disorder in women of reproductive age (Franks, 1995, 2008; Knochenhauer *et al*., 1998; Diamanti-Kandarakis *et al*., 2008; Asunción *et al*., 2000; Azziz, 2004; Wood *et al*., 2007; Toulis *et al*., 2009). It is characterized by a clustering of hyperandrogenism, hyperinsulinemia, hypersecretion of LH, menstrual dysfunction, hirsutism, infertility and pregnancy and neonatal complications (Franks, 1995; Moran and Teede, 2009; Stubbs *et al*., 2007; Toulis *et al*., 2009). Women with PCOS undergoing IVF treatment have been well-described (Ludwig *et al*., 1999; Legro, 2001; Mulders *et al*., 2003; Heijnen *et al*., 2006; Sahu *et al*., 2008). Although PCOS patients are typically characterized by producing an increased number of oocytes, they are often of poor quality, leading to lower fertilization, cleavage and implantation rates, and a higher miscarriage rate (Sengoku *et al*., 1997; Ludwig *et al*., 1999; Mulders *et al*., 2003; Heijnen *et al*., 2006; Weghofer *et al*., 2007; Sahu *et al*., 2008; Boomsma *et al*., 2008). This evidence raises the issue that poor oocyte and embryo quality may contribute to increased aneuploidy rates (Munné *et al*., 1995; Gianaroli *et al*., 2003, 2007). However, recent data suggest that women with PCOS yield higher numbers of oocytes and produce more euploid embryos in IVF, but still result in lower pregnancy and increased miscarriage rates, which are not genetically associated with an increased risk for embryonic aneuploidy (Weghofer *et al*., 2007). Hence, other factors, aside from chromosomal factors, are most likely associated with the significantly increased risk for pregnancy loss in patients with PCOS (Sagle *et al*., 1988; Carmina and Lobo, 1999; Wood *et al*., 2007; Weghofer *et al*., 2007). Impaired oocyte maturation and embryonic developmental competence in PCOS women is possibly linked with abnormal endocrine/paracrine factors, metabolic dysfunction and alterations in the intrafollicular microenvironment during folliculogenesis and follicle maturation (Franks *et al*., 2002; Dumestic *et al*., 2007b; Dumesic and Abbott, 2008; Wood *et al*., 2007). Therefore, a better understanding of how PCOS is related to abnormalities in extra- and intra-ovarian factor (Fig. 1, Table I) and their impact on granulosa cell (GC)–oocyte interactions, oocyte maturation and potential embryonic developmental competence, will be crucial to improving fertility and optimizing clinical stimulation, thus enhancing pregnancy outcomes in women with PCOS undergoing IVF treatment.

Methods

For this review, we searched the database MEDLINE (1950 to January 2010) and GOOGLE for all full texts and/or abstract articles published in English; our own unpublished data were taken into account as well. Search terms included 'oocyte', 'embryo', 'oocyte and embryo', 'oocyte and embryo quality', 'oocyte quality', 'embryo quality', 'fertilization', 'oocyte aneuploidy', 'embryo aneuploidy', 'oocyte abnormality', 'embryo abnormality', 'clinical issue', 'laboratory issue', 'IVF outcome', 'follicle fluid', 'follicle fluid hormone', 'follicle fluid and oocyte', 'follicle fluid and embryo', 'folliculogenesis', 'extra- and intra-ovarian factors', 'follicular fluid factors' and 'growth factors' in PCOS. This search resulted in 1596 papers. Upon screening the results for applicable titles and/or abstracts, only articles correlating to PCOS and its relatives were selected for this review. In addition, we hand-searched references of relevant reviews, and conference abstracts, and included ongoing studies to locate other potentially eligible materials.

Extra-ovarian factors

Human folliculogenesis and follicle maturation are complicated developmental processes through which a mature follicle is differentiated from primordial follicles, yielding one mature follicle that is eventually selected to ovulate, releasing a mature oocyte. This developmental process can be disrupted by abnormal extra-ovarian endocrine factors, resulting in ovarian dysfunction. Complex endocrine disorders, such as FSH deficiency, hypersecretion of LH, hyperandrogenemia and hyperinsulinemia with insulin resistance, are responsible for the pathogenesis of PCOS, consequently increasing the risks of impaired oocyte developmental competence, implantation failure and miscarriage (Van der Spuy and Dyer, 2004; Dumesic *et al*., 2007a; Dumesic and Abbott, 2008; Boomsma *et al*., 2008).

FSH deficiency

FSH stimulates follicular growth and recruitment of immature follicles from the ovary. FSH is the major survival factor during folliculogenesis, when there is a delicate balance between recruitment and atresia of follicles. Human antral follicles between 2 and 5 mm become responsive to FSH, whereas slightly larger follicles between 6 and 8 mm acquire aromatase activity and potentially increase the estradiol (E_2) levels (Dumesic and Abbott, 2008). With the concomitant rise in E_2 and inhibin B, FSH levels then decline in the late follicular phase, and eventually only the most advanced and mature follicle is selected to proceed to ovulation. At the end of the luteal phase, there is a slight rise in the FSH level, which is very important in initiating the next ovulatory cycle (Erickson and Shimasaki, 2001; Padhy *et al*., 2009). In contrast, PCOS patients show lower serum FSH levels as compared with normal cycles (Hillier, 1994). Consequently, FSH deficiency results in an increased accumulation of antral follicles between 2 and 8 mm (Franks *et al*., 2000, 2008). Clearly, the high number of smaller follicles indicates many have undergone premature arrest and failed to become the dominant follicle (Franks *et al*., 2008; Padhy *et al*., 2009). However, the developmental competence of oocytes collected from women with PCOS is normal, potentially leading to similar fertilization and normal cumulative pregnancy rates

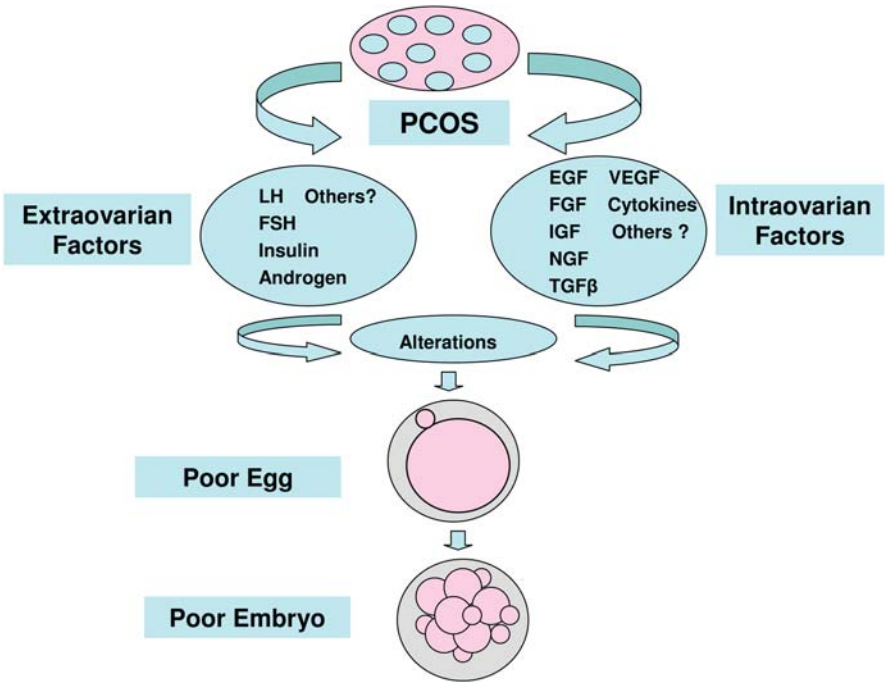

Figure 1 Intra-and extra-ovarian factors associated with the PCOS pathology that negatively affect oocyte and subsequent embryo quality.

(Hardy *et al*., 1995; Ludwig *et al*., 1999; Jabara and Coutifaris, 2003; Heijnen *et al*., 2006; Franks *et al*., 2008).

PCOS patients undergoing IVF commonly demonstrate elevated E_2 levels, combined with a significantly higher number of oocytes retrieved, lower number of high-quality oocytes, poor fertilization rates, increased embryonic fragmentation, decreased percentage of blastocyst formation and lower impanation rates (Cano *et al*., 1997a, b; Urman *et al*., 2004). High E_2 levels in PCOS patients may be detrimental to oocyte maturation and embryonic development (Hardy *et al*., 1995). In addition, recovery of immature oocytes followed by *in vitro* maturation (IVM) is a potentially useful treatment option for women with PCOS-related infertility. As an alternative approach, minimal or mild ovarian stimulation with FSH before oocyte collection has been applied in PCOS patients (Chian, 2004). Immature oocytes are then cultured in complex IVM culture medium plus 75 mIU/ml FSH + LH 75 mIU/ml for 24–48 h. ICSI is performed for mature oocytes. Despite the elevated number of immature oocytes obtained from PCOS patients with declining serum FSH levels (Dumesic *et al*., 2007b; Franks *et al*., 2008), oocyte maturation *in vitro* induced by extrinsic FSH and cumulus cell (CC)–oocyte interactions are crucial for the acquisition of oocyte developmental potential (Wynn *et al*., 1998; Dumesic *et al*., 2007b). Consequently, oocytes become fertilized embryos and potentially develop into the blastocyst stage (De La Fuente, 2006; Dumesic *et al*., 2007b). Results suggest that the cumulative pregnancy rate by IVM treatment in women with PCOS is comparable with that of other PCOS women undergoing conventional IVF (Child *et al*., 2001; Cha *et al*., 2005; Söderström-Anttila *et al*., 2005). However, recent studies have suggested that IVM has deleterious effects on the spindle organization and chromosomal configuration of oocytes from PCOS patients (Li *et al*., 2006; Navarro *et al*., 2007, 2009; Nichols *et al*., 2010), possibly explaining the reduced developmental competence of oocytes matured *in vitro*, compared with those matured *in vivo*. This may possibly contribute to the decline in the overall clinical outcome observed after IVM treatment (Li *et al*., 2006; Navarro *et al*., 2007, 2009).

Hypersecretion of LH

Women with PCOS typically have tonic hypersecreton of LH during the follicular phase of their cycles (Balen *et al*., 1993; Cano *et al*., 1997a, b; van der Spuy and Dyer, 2004). High LH levels have been associated with significant decreases in oocyte maturation and fertilization rates, and impaired embryo quality, consequently resulting in impaired pregnancy rates, and higher miscarriage rates (Adams *et al*., 1985; Stanger and Yovich, 1985; Hombur and Jacobs, 1989; Regan *et al*., 1990; Sengoku *et al*., 1997; Ludwig *et al*., 1999; Jabara and Coutifaris, 2003; Urman *et al*., 2004; van der Spuy and Dyer, 2004; Santos *et al*., 2010). Hypersecretion of LH during folliculogenesis may suppress FSH function, resulting in abnormal GC function by promoting premature GC luteinization and follicular aresia in small antral follicles from women with PCOS, causing premature oocyte maturation via inhibition of oocyte maturation inhibitors (Tesarik, 2003; van der Spuy and Dyer, 2004; Dumesic *et al*., 2007b; Franks *et al*., 2008), which all impair the quality of both oocyte and embryo (Tarlatzis and Grimbizis, 1997; Dumesic *et al*., 2002). LH may also activate premature meiotic processes by damaging the oocyte nucleus, leading to apoptosis via a receptor-coupled signal transduction system (Yoshimura and Wallach, 1987; Kurzawa *et al*., 2008). Disruption of the endocrine control of meiosis, resulting in impaired extrusion of the first polar body, may compromise the

Table I Factors in serum and follicular fluid of patients with PCOS: impact on quality of oocyte and embryo, fertilization and outcome of pregnancy.

Factors	Serum level	FF level	Oocyte quality	Fertilization rate	Embryo quality	Pregnancy rate	References
Activin	↓	↓					Norman *et al.* (2001), Erickson *et al.* (1995)
Anti-Müllerian hormone	↑	↑	↑ or ↓	↑ or ≈ or ↓	↑ or ≈	↑ or ≈	Fallat *et al.* (1997), Wang *et al.* (2007a, b), Mashiach *et al.* (2010), Desforges-Bullet *et al.* (2010)
Epidermal growth factor		↑					Volpe *et al.* (1991), Almahbobi *et al.* (1998), Artini *et al.* (2007)
Fibroblast growth factor	↓ or ↑	↓ or ↑	≈ or ↓	≈	≈		Hammadeh *et al.* (2003), Artini *et al.* (2006)
Follistatin	↑	↑					Erickson *et al.* (1995), Norman *et al.* (2001), Eldar-Geva *et al.* (2001)
Brain-derived neurotrophic factor		↑					Johnstone *et al.* (2008), Buyuk and Seifer (2008)
Bone morphogenetic protein-15		↑	↑	↑	↑		Wu *et al.* (2007a, b)
Estradiol	↓	↓	↓	↓	↓		Berker *et al.* (2009), Amato *et al.* (2003)
Follicular fluid meiosis-activating sterol		↑	↑	≈	≈		Bokal *et al.* (2006)
Growth differentiation factor-9		↓	↓				Zhao *et al.* (2010)
Homocysteine	↑	↑	↓	↓	↓		Nafiye *et al.* (2010), Berker *et al.* (2009)
Insulin-like growth factor-1 & 2	↓	↓	↓				Schoyer *et al.* (2007), Barreca *et al.* (1996), Eden *et al.* (1988)
IGF binding proteins	↑	↑	↓				Cataldo and Giudice (1992), Schoyer *et al.* (2007)
Interleukin 12		↓	↓	↓	↓		Gallinelli *et al.* (2003)
Interleukin 13		↑	↓	↓	↓		Gallinelli *et al.* (2003)
Inhibin A&B		↓ or ≈	≈				Welt *et al.* (2005), Magoffin and Jakimiuk (1998)
Corticotrophin-releasing hormone		↓	↓				Mastorakos *et al.* (1994)
Leptin	↑	↑	↓	↓	↓	↓	Mantzoros *et al.* (2000), Georgios *et al.* (2005), Li *et al.* (2007)
Leukemia inhibitory factor		↓	↓	↓	↓	↓	Lédée-Bataille *et al.* (2001)
Malondialdehyde	↑	↑	↓	↓	↓		Yildirim *et al.* (2007), Berker *et al.* (2009), Chattopadhayay *et al.* (2010)
Matrix metalloproteinase 2/9		↑ or ≈					Shalev *et al.* (2001), Lahav-Bratz *et al.* (2003)
Nerve growth factor		↑ or ↓					Dissen *et al.* (2009); Buyuk and Seifer (2008)
Renin	↓	↓	↑	↑	↑		Bokal *et al.* (2003, 2004, 2005)
Resistin	≈	≈	≈	≈		≈	Seow *et al.* (2005), Lu *et al.* (2005)
Reactive oxygen species		↑	↓	↓	↓	↓	Chattopadhayay *et al.* (2010), Samanta *et al.* (2008)
Soluble Fas	↓	↓	↓				Onalan *et al.* (2005)
sFas Ligand	↑	↑	↓				Onalan *et al.* (2005)

Continued

Table I *Continued*

Factors	Serum level	FF level	Oocyte quality	Fertilization rate	Embryo quality	Pregnancy rate	References
Superoxide dismutase	↓ or ≈	↓ or ≈					Sabatini *et al.* (2000), Bausenwein *et al.* (2010)
Total antioxidant capacity	↓	↓	↓	↓	↓	↓	Chattopadhayay *et al.* (2010)
Testosterone	↑	↑	↓				Brzynski *et al.* (1995), Teissier *et al.* (2000)
Tissue inhibitor of metalloproteinase-1 & 2		↓ or ≈					Lahav-Bratz *et al.* (2003), Shalev *et al.* (2001)
Tumor necrosis factor α	↑	↑	↓	↓	↓	↓	Amato *et al.* (2003), Wu *et al.* (2007a, b), Kim *et al.* (2009)
Vascular endothelial growth factor	↓ or ↑	↓ or ↑	↓	↓ or ≈	↓	↓	Bokal *et al.* (2004, 2005, 2009), Artini *et al.* (2006, 2009)
Visfatin	↑	≈					Plati *et al.* (2009)

All data are as compared with controls (patients without PCOS).↑, increases or positive impact; ↓, decreases or negative impact; ≈, similar; blank, no data.

chromosomal normality of oocytes (Sengoku *et al.*, 1997), possibly contributing to embryonic aneuploidy in women with PCOS (Weghofer *et al.*, 2007). Errors in embryogenesis stemming from abnormal and premature oocyte exposure to increased LH stimulation may explain the elevated miscarriage rate in PCOS patients (Balen *et al.*, 1993; Urman *et al.*, 2004).

Hyperandrogenemia

Hyperandrogenemia is a common disorder in PCOS; it is mutifactorial in origin, typically attributed to the ovary with substantial contributions from an adrenal source, and to a lesser extent adipose tissues (van der Spuy and Dyer, 2004; Nisenblat and Norman, 2009). Elevated free circulating levels of bioactive androgen results from either direct increases of ovarian production or an inhibition of hepatic synthesis of sex hormone-binding globin in PCOS patients with insulin resistance (Balen *et al.*, 1995; van der Spuy and Dyer, 2004; Nisenblat and Norman, 2009). Increased androgen concentrations in the follicular fluid (FF) are associated with elevated serum LH levels, which may block dominant follicle development and cause follicular arrest and degeneration (Billig *et al.*, 1993; Kurzawa *et al.*, 2008). It has been suggested that high levels of androgen may have a negative impact on oocyte developmental competence (Brzynski *et al.*, 1995; Teissier *et al.*, 2000; Jabara and Coutifaris, 2003). Incubation of the oocyte with androgen *in vitro* is associated with decreased oocyte maturation rates (Tesarik and Mendoza, 1995). Data from an *in vitro* model suggest that testosterone exerts a strong inhibition of meiotic maturation and embryonic development in CC-free mouse oocytes, compared with CC-enclosed oocytes; this demonstrates that CCs can protect oocytes via local aromatase activity in human (Laufer *et al.*, 1984; Dumesic *et al.*, 2007b) and mice (Anderiesz and Trounson, 1995). Such a CC function plays an important role in PCOS folliculogenesis, since small PCOS follicles are hyperandrogenic (Eden *et al.*, 1990; Dumesic *et al.*, 2007b) owing to intrinsically raised androgen biosynthesis by theca cells (Nelson *et al.*, 2001). Further studies have suggested that elevated testosterone, either directly or indirectly, decreases the rates of IVM, fertilization and embryonic development (Dumesic *et al.*, 2007b; Patel and Carr, 2008). The mechanism of testosterone activity within the oocyte may be related to decreased calcium oscillations, consequently inhibiting oocyte cytoplasmic maturation, with effects on meiotic maturation (Tesarik and Mendoza, 1995, 1997; Jabara and Coutifaris, 2003). In addition, elevated testosterone concentrations are associated with higher miscarriage rates in women with PCOS (van der Spuy and Dyer, 2004), suggesting that androgens may have a detrimental effect on folliculogenesis and endometrial function (Okon *et al.*, 1998; Tuckerman *et al.*, 2000).

Hyperinsulinemia

PCOS is an endocrine–metabolic disorder, closely tied to insulin resistance and a compensatory hyperinsulinemia. Metformin is the drug which has been studied most, and is administered to reduce fasting insulin, LH and free testosterone level, in an effort to restore menstrual cyclicity and fertility (Tang *et al.*, 2010). It has been reported that insulin resistance is related to an increased miscarriage rate (Craig *et al.*, 2002); several studies have suggested that metformin can effectively reduce pregnancy loss in women with PCOS (Glueck *et al.*, 2001; Jakubowicz *et al.*, 2002; Kjotrod *et al.*, 2004; Galal and Mitwally, 2009). Hyperinsulinemia may have preferentially impaired oocyte developmental competence, resulting in reduced rates of fertilization, embryonic development and implantation in PCOS patients with obesity (Hamilton-Fairley *et al.*, 1992; Cano *et al.*, 1997a, b; Wang *et al.*, 2001; Wijeyaratne *et al.*, 2002; Jabara and Coutifaris, 2003; Dumesic *et al.*, 2002, 2007b; Dumesic and Abbott, 2008; Palep-Singh *et al.*, 2007; Tian *et al.*, 2007; Boomsma *et al.*, 2008). Data from *in vitro* cell culture models suggest that co-incubation of insulin and FSH with mouse (Eppig *et al.*, 1998) and bovine (Galal and Mitwally, 2009) oocytes promotes FSH-induced up-regulation of GC LH receptor mRNA expression (Dumesic *et al.*, 2002; Tao and Yan, 2005; Diamanti-Kandarakis, 2008), inhibiting FSH stimulation of aromatase activity (Galal and Mitwally, 2009), thus reducing the percentage of fertilized oocytes that develop into blastocysts (Eppig *et al.*, 1998; Dumesic *et al.*, 2002, 2007b). Insulin may induce local androgen production, which results in oocytes of lower quality, post-maturity (Cano *et al.*, 1997a, b). At the molecular level, insulin binds to its receptor, localized on GC and theca cells, and oocytes, to stimulate follicle

recruitment (Dumesic *et al*., 2002, 2007b; Kezele *et al*., 2002), consequently altering expression of multiple genes involved in meiotic/mitotic spindle dynamics and centrosome function in PCOS ooctyes (Wood *et al*., 2007). This indicates that insulin may be an important mediator of oocyte developmental competence via a ligand-receptor regulating system (Dumesic *et al*., 2007b).

Intra-ovarian factors

Ovarian folliculogenesis is regulated by a fine balance between extra and intra-ovarian factors (Artini *et al*., 2007). Oogenesis is profoundly dependent upon intra-ovarian factors, in particular follicle fluid factors (FFFs) (Andreani *et al*., 1996; Hsieh *et al*., 2009; Padhy *et al*., 2009), which are positively related to levels of these factors in serum (Table I). Any imbalance or dysfunction between extra- and intra-ovarian factors may result in abnormal folliculogenesis and oogenesis disorder (Frank *et al*., 2002, 2008; Artini *et al*., 2007). Recent studies suggest that the main FFFs implicated in polycystic ovary folliculogenesis are members of the growth factor families, cytokines, inhibins and others (Franks *et al*., 2002; Artini *et al*., 2007; Diamanti-Kandarakis, 2008). Furthermore, a series of different serum factors, coupled with the intrafollicular fluid microenvironment, may directly impair oocyte developmental competence, should their balance be altered (Yen *et al*., 1993; Andreani *et al*., 1996; van der Spuy and Dyer, 2004; Artini *et al*., 2007; Padhy *et al*., 2009); this would consequently have a negative impact on the fertilization, embryonic development and outcome of pregnancy in PCOS patients (Table I and Fig. 1).

Epidermal growth factor family

Epidermal growth factor (EGF) is a soluble growth factor that plays an important role in the regulation of cell growth, proliferation and differentiation when bound to its receptor, EGFR (ErbB1, ErbB2-4; Hsieh *et al*., 2009). In the human ovary, EGF is found in the FF, regulating follicular development and oocyte meiotic maturation competence via EGFR signaling transduction system in the CCs (Westergaard and Andersen, 1989; Almahbobi *et al*., 1998; Jamnongjit *et al*., 2005; Hsieh *et al*., 2009). IVM studies show that exposure of the cumulus–oocyte complex (COC) to EGF stimulates CC expansion and improves the nuclear and cytoplasmic maturation of oocytes from the metaphase I (MI) to metaphase II (MII) stage in both humans and other mammals (Goud *et al*., 1998; Smitz *et al*., 1998; De La Fuente *et al*., 1999), significantly facilitating fertilization and embryo development (Singh *et al*., 1997; Goff *et al*., 2001). Other studies suggest that FF EGF levels have an inverse correlation with oocyte maturation (Hofmann *et al*., 1990; Das *et al*., 1992; Ozornek *et al*., 1999; Hsieh *et al*., 2009). In women with PCOS, FF EGF levels are higher than those of normally ovulating women (NOW), which may suggest the involvement of EGF in the maintenance of PCOS (Volpe *et al*., 1991; Artini *et al*., 2007). EGF inhibits estrogen synthesis in GCs, which may explain why EGF blocks antral follicle growth and results in follicular arrest in PCOS patients (Artini *et al*., 2007). Therefore, it is hypothesized that a disruption in the regulatory mechanisms of EGF synthesis and/or physiological function mediated by EGFR may cause anovulatory infertility in women with PCOS (Almahbobi and Trounson, 1996; Almahbobi *et al*., 1998). Whether an elevated level of EGF in FF is correlated to oocyte quality and embryonic developmental competence is still unclear.

In addition, EGF-like factors, such as amphiregulin, epiregulin and betacellulin, are reportedly involved in oocyte maturation through autocrine and paracrine mechanisms (Ashkenazi *et al*., 2005; Shimada *et al*., 2006; Tse and Ge, 2009); however, the physiological function of EGF-like factors in PCOS remains unknown.

Fibroblast growth factor family

Fibroblast growth factors (FGFs) are a group of polypeptides that play a fundamental role in development, cell growth, tissue repair and transformation (Hammadeh *et al*., 2003). They are expressed in GC and theca cells of growing follicles, and are considered to be physiological regulators of FSH action (Artini *et al*., 2006, 2007); this may suggest a role for FGF in oocyte maturation by affecting surrounding follicular GC and theca cells (Skinner, 2005; Artini *et al*., 2007). A previous study shows that FGF levels in the serum and FF are lower in PCOS patients in comparison to patients with endometriosis and tubal factors (Hammadeh *et al*., 2003). In contrast, another research group reported that FGF concentrations are increased in the FF and serum of PCOS patients when compared with controls, leading to an inverse correlation with oocyte maturity (Artini *et al*., 2006, 2007): this supports speculation that FGF contributes to alterations in the intra-follicle environment, resulting in arrest of follicle development in patients with PCOS (Artini *et al*., 2007). Therefore, FGF alterations in the FF and serum remain controversial; the impact of EGF on oocyte maturation and embryonic development requires further elucidation in PCOS patients.

Insulin-like growth factor family

Insulin-like growth factors (IGFs) are multifunctional polypeptides with insulin-like activity. IGFs are part of a complex system used by cells to communicate with their physiological environment. This complex system consists of two surface-receptors (IGF1R and IGF2R), two receptor ligands (IGF-I and IGF-II), six high-affinity IGF binding proteins (IGFBP 1-6) and their specific proteases (Adashi, 1993; Frattali and Pessin, 1993; Yen *et al*., 1993; Erickson and Shimasaki, 2001; Artini *et al*., 2007).

Insulin-like growth factor-I/II and IGF binding proteins

IGFs and their binding proteins, IGFBPs, have important regulatory functions in ovarian follicular development (Yen *et al*., 1993; Artini *et al*., 2007). Circulating IGFs are produced in the liver, local IGF-I is secreted by theca cells whereas IGF-II is synthesized by GCs, and IGFBPs are present in the FF and expressed by GCs and theca cells (Yen *et al*., 1993; Erickson and Shimasaki, 2001; Artini *et al*., 2007). Although how IGFs are involved in the pathogenesis of PCOS remains unknown, the excess insulin concentrations and alterations in IGFs expression may be implicated (Yen *et al*., 1993). One recent report suggests that the FF IGF-I levels in PCOS women are elevated, although IGF-II and IGFBP-1 levels are lower than NOW (Artini *et al*., 2007). However, FF IGFBP-2 and -4 levels are significantly greater (Yen *et al*., 1993; Kwintkiewicz and Giudice, 2009); in contrast, IGFBP-1 is lower in PCOS patients, leading to follicular arrest (Artini *et al*., 2007). This evidence suggests that an altered IGF system is directly correlated to the oligo-ovulatory disorder of PCOS women (Kwintkiewicz and Giudice, 2009).

Women with PCOS have a higher FF IGFBP-3, but unaltered FF IGF-I levels (Amato *et al*., 1999). Research shows the levels of IGF-I, IGF-II and IGFBP-3 in mature follicles to be comparable between PCOS patients and controls; however, IGF-I levels in immature follicles in PCOS patients is decreased during ovarian stimulation, and this is associated with the generation of immature oocytes (Eden *et al*., 1988; Rabinovici *et al*., 1990; Franchimont *et al*., 1994; Pellegrini *et al*., 1995; Barreca *et al*., 1996; Dragisic *et al*., 2006; Schoyer *et al*., 2007), At the same time, IGFBP-3 levels are increased during stimulation, resulting in a greater likelihood of achieving pregnancy in PCOS patients (Schoyer *et al*., 2007). In infertile IVF patients, the ratio of IGF-1/IGFBP-1 in the serum and FF is significantly increased in women who become pregnant, highlighting the importance of oocyte quality and maturity during ovarian stimulation for IVF (Jimena *et al*., 1992; Artini *et al*., 1994; Kawano *et al*., 1997; Oosterhuis *et al*., 1998; Fried *et al*., 2003). Furthermore, results from *in vitro* culture models demonstrate that IGF-I can significantly increase embryonic development and blastocyst formation (Lighten *et al*., 1998; Liu *et al*., 1999; Fried *et al*., 2003). Hence, study of FF proteins may help to elucidate the roles of IGFs in GC function, meiotic maturity, oocyte chromosomal normality and embryonic developmental competence in PCOS patients.

Neurotrophin growth factor family

Brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), NT-3 and NT-4/5 are major members of the neurotrophin (NT) family of growth factors that are involved in development of the central and peripheral nervous systems (Levi-Montalcini, 1987; Snider, 1994; Buyuk and Seifer, 2008). NTs are not only involved in the nervous system, but also act on the ovaries of humans and other mammals (Seifer *et al*., 2002a, b, 2003; Buyuk and Seifer, 2008). NTs play a fundamental role in folliculogenesis and cytoplasmic competence of the oocyte (Buyuk and Seifer, 2008). Data from research using *in vitro* animal models suggest that co-incubation with BDNF promotes nuclear and cytoplasmic maturation of the oocyte, which are essential processes for successful oocyte and preimplantation embryo development (Da Silva *et al*., 2005; Kawamura *et al*., 2005). Evidence from some studies shows that increased FF BDNF and NGF levels are closely related to the pathology of women with PCOS (Bai *et al*., 2004; Johnstone *et al*., 2008; Dissen *et al*., 2009). Another report found that FF BDNF and NT-3 levels are increased, but FF NGF is decreased, in women with PCOS (Buyuk and Seifer, 2008) which may be indicative of the differential status of follicles in PCOS patients. Therefore, NT mechanisms in POCS pathogenesis, especially their impact on oocyte and embryo developmental competence, need further clarification at different stages of follicle development.

Transforming growth factor-β family

Among the many intra-ovarian factors, particular members of the transforming growth factor (TGF)-β family play an important biological role in follicle growth and oocyte development. These family members include anti-Müllerian hormone (AMH)/Müllerian inhibiting substance (MIS), activin, follistatin, inhibins, bone morphogenetic protein (BMP)-9 and growth differentiation factor (GDF)-9 (PieK *et al*., 1999; Artini *et al*., 2007; Dumesic *et al*., 2007b; Dumesic and Abbott, 2008). Under different physiological conditions, TGF-β family members may either promote or block ovarian follicle growth and/or differentiation of the GC–oocyte complex, which is also related to the pathogenesis of PCOS (van der Spuy and Dyer, 2004; Diamanti-Kandarakis, 2008; Dumesic and Abbott, 2008).

Anti-Müllerian hormone/Müllerian inhibiting substance

AMH, also known as Müllerian inhibiting factor (MIF), Müllerian inhibiting hormone (MIH) or MIS, is a homodimeric glycoprotein with a molecular weight of 140 kDa (Di Clemente *et al*., 2003; Artini *et al*., 2007). It inhibits the development of the Müllerian ducts in the male embryo (Behringer, 1994). AMH is expressed by GCs within ovaries of women of reproductive age, controlling the formation of primary follicles by inhibiting excessive follicular recruitment by FSH and therefore plays an important role in folliculogenesis (Weenen *et al*., 2004; Sadeu and Smitz, 2008). Some studies have demonstrated that AMH levels reflect some aspects of ovarian function, making AMH levels a potential marker for assessing conditions such as PCOS and premature ovarian failure (Visser *et al*., 2006; Sir-Petermann and King, 2007; Diamanti-Kandarakis, 2008; Dumesic and Abbott, 2008; Marca *et al*., 2009). Women with PCOS have elevated serum and FF AMH levels versus those of normal controls (Pigny *et al*., 2003; Laven *et al*., 2004; Artini *et al*., 2007), which is closely associated with increased development of antral follicles and follicular arrest in PCOS patients (Artini *et al*., 2007; Das *et al*., 2008; Diamanti-Kandarakis, 2008; Franks *et al*., 2008). Elevated AMH serum levels are directly correlated with increased testosterone and/or LH levels in women with PCOS, and profoundly impairing oocyte developmental competence and embryo quality (Tarlatzis and Grimbizis, 1997; Dumesic *et al*., 2002, 2007b; Patel and Carr, 2008; Franks *et al*., 2008). Also, elevated FF AMH concentrations in women with PCOS are linked to an increased percentage of immature oocyte and lower fertilization rates when compared with women with endometriosis or pelvic adhesions (Fallat et a., 1997); this is supported by evidence from the rat model as well (Takahashi *et al*., 1986). Recent complementary investigation suggests that increased FF AMH in women with PCOS may have harmful consequences on oocyte quality and maturation, via an unclear molecular mechanism, but does not have an effect on pregnancy rates (Desforges-Bullet *et al*., 2010).

In a contrasting study conducted among women with PCOS, results suggest that fertilization, implantation and clinical pregnancy rates are significantly better in the group with the highest FF AMH concentration than in any group with a lower concentration (Pabuccu *et al*., 2009). Additional reports reveal that women with PCOS who have lower FF AMH levels have similar rates of oocyte maturation, fertilization and embryonic development compared with NOW (Wang *et al*., 2007a, b; Mashiach *et al*., 2010). However, recent evidence suggests that FF AMH concentrations are only strongly and positively associated with oocyte quality and implantation rates, but not rates of oocyte fertilization, embryo cleavage and embryo morphology in NOW (Ebner *et al*., 2006; Fanchin *et al*., 2007; Marca *et al*., 2009). Still, others demonstrate that lower AMH levels are associated with poor oocyte quality, as supported by decreased fertilization and embryonic developmental rates, and increased miscarriage rates in IVF patients (Lekamge *et al*., 2007) Therefore, AMH may directly affect cytoplasmic maturation of the oocytes. Based upon all of the above

studies, variation in levels of AMH may indicate different physiological conditions during follicle development and oocyte maturation. Hence, AMH may not be a valuable predictor for success in NOW and women with PCOS undergoing assisted reproduction.

Activin, follistatin and inhibin

The activins, follistatin (FS) and inhibins are polypeptides which were originally isolated and characterized from ovarian FF. FS is an activin/inhibin binding protein produced by ovarian GCs, believed to act in an autocrine/paracrine manner to regulate growth and differentiation (Shimonaka *et al*., 1991; Findlay, 1993; Erickson *et al*., 1995); over-expression of FS has been associated with increased arrest of follicular development and decreased oocyte developmental competence (Erickson *et al*., 1995; Norman *et al*., 2001). Activins are preferentially secreted by these smaller follicles, promoting follicular development by increasing the GC response to FSH stimulation, decreasing androgen synthesis and enhancing oocyte maturation. Inhibins on the other hand, are produced by the dominant follicle and stimulate theca cell androgen production for E_2 synthesis (Schwall *et al*., 1990; Klein *et al*., 2000; Knight and Glister, 2001; Dumesic *et al*., 2007b). In NOW, studies suggest that FF levels of inhibin A, inhibin B and activin A reflect changes in follicle size, but are not independent markers of the oocyte's ability to achieve fertilization and pregnancy (Fried *et al*., 2003; Wen *et al*., 2006).

An early study failed to demonstrate any correlation between inhibin A and B concentration in the FF and oocyte quality and fertilization rates (Lau *et al*., 1999). IVF patients with high FF inhibin A and B levels, measured on the day of oocyte retrieval, have better oocyte maturity and fertilization rates, and higher pregnancy rates (Dzik *et al*., 2000; Ocal *et al*., 2004). Another study reported that inhibin B levels in the FF are significantly correlated to embryo quality, but not oocyte quality (Change *et al*., 2002). Interestingly, no differences were found in the levels of FS and activin A in the FF from normal, atretic or polycystic ovaries (Erickson *et al*., 1995).

Increased FS/activin ratios (high FS and low activin A) are well-known contributors to the pathophysiology of PCOS (Eldar-Geva *et al*., 2001; Norman *et al*., 2001); both proteins have an effect on oocyte maturity and developmental competence, with activin enhancing post-fertilization development, and FS blocking this function (Norman *et al*., 2001). Elevated inhibin B levels are closely related to an elevated risk of developing PCOS (Magoffin and Jakimiuk, 1997; Anderson *et al*., 1998; Lockwood *et al*., 1998). In addition, studies have shown that inhibin A and B levels are significantly reduced in the FF of women with PCOS, when compared with FF levels of size-matched follicles from NOW (Lambert-Meserlian *et al*., 1997; Welt *et al*., 2005). Therefore, activin, FS and inhibins bring about intra-ovarian actions through paracrine/autocrine systems, playing an important role in maintaining folliculogenesis; their imbalance may be directly correlated to the pathogenesis of PCOS, consequently impairing oocyte maturity, embryo quality and pregnancy outcome.

Growth differentiation factor-9 and bone morphogenetic protein-15

GDF-9 and BMP-15 (also called as GDF-9b) are two closely related members of the TGF-β family of proteins and are highly expressed in growing and full grown oocytes (Teixeira Filho *et al*., 2002; Gilchrist *et al*., 2008; Chen *et al*., 2009). BMP-15 and GDF-9 play fundamental roles in regulating CC functions through the processes of mitosis, proliferation, apoptosis, luteinization, metabolism and expansion through mitogenic signaling transduction mechanisms (Erickson and Shimasaki, 2001; Teixeira Filho *et al*., 2002; van der Spuy and Dyer, 2004; Gilchrist *et al*., 2008; Chen *et al*., 2009). Data from *in vitro* models demonstrate that co-incubation of COC with either BMP-15 or GDF-9 substantially promotes oocyte maturation and enhances blastocyt production, as well as increases the total number of cells in the trophectoderm (Hussein *et al*., 2006) and inner cell mass of mouse embryos (Yeo *et al*., 2008). Following embryo transfer in mice, the rate of fetal survival almost doubles after exposure to BMP-15 or GDF-9, but no differences could be detected in implantation rates (Yeo *et al*., 2008). Importantly, both GDF-9 and BMP-15 are required for folliculogenesis in humans and their abnormal expression may be related to female infertility (Juengel *et al*., 2002; Teixeira Filho *et al*., 2002; Shimasaki *et al*., 2004; Artini *et al*., 2007; Wu *et al*., 2007a, b; Gilchrist *et al*., 2008), including increased correlations with PCOS pathologies (Franks *et al*., 2002; Teixeira Filho *et al*., 2002; van der Spuy and Dyer, 2004; Ciepiela *et al*., 2007; Dumesic *et al*., 2007b, Dumesic and Abbott, 2008; Gilchrist *et al*., 2008; Zhao *et al*., 2010). In infertile women, elevated FF BMP-15 levels are positively correlated with improved oocyte quality and higher rates of fertilization and embryonic development, suggesting that BMP-15 may be a good indicator of oocyte maturity and fertilization ability (Wu *et al*., 2007a, b). A recent study demonstrates that the expression of GDF-9 and BMP-15 tended to be higher in PCOS patients when compared with a control group, and thus may be involved in PCOS follicular dysplasia (Zhao *et al*., 2010). GDF-9 expression in CCs is lower in PCOS patients, which may lead to premature luteinization and decreased oocyte developmental competence and luteal generation (Takebayashi *et al*., 2000; Artini *et al*., 2007): this may also be correlated to elevated miscarriage rates in women with PCOS (Zhao *et al*., 2010). Therefore, the expression of BMP-15 and GDF-9 in both oocytes and CCs may provide valuable support for the ability to regulate the follicular microenvironment during the oocyte maturation process. Further study on the role of BMP-15 or GDF-9 during follicle growth and oocyte meiotic maturation will have important implications in understanding those factors that regulate the mechanisms behind the pathogenesis of PCOS, and help to improve IVM methods for oocytes from women with PCOS.

Vascular endothelial growth factor family

Vascular endothelial growth factor (VEGF) is a homodimeric glycoprotein belonging to the VEGF/platelet-derived growth factor family (Artini *et al*., 2007). In the ovary, VEGF is expressed in GCs and theca cells, but rarely in stroma cells (Artini *et al*., 2007) and is also present in the FF (Artini *et al*., 1998; Van Blerkom, 2000; Stouffer *et al*., 2001; Ocal *et al*., 2004). VEGF exerts its actions by binding to one of three receptors, VEGFR-1/Flt-1, VEGFR-/KDR/Flk-1 or VEGFR-3/Flt-4, functioning via the signal transduction system (De Vries *et al*., 1992; Terman *et al*., 1992; Artini *et al*., 2007, 2009). VEGF plays an important role in angiogenesis, follicular vascularization and intrafollicular oxygenation, consequently impacting follicular maturation, oocyte quality, fertilization and embryo developmental competence (Itskovitz *et al*., 1991; Van Blerkom *et al*., 1997; Agrawal *et al*.,

1998, 2002; Loret de Mola *et al*., 1999; van der Spuy and Dyer, 2004; Bokal *et al*., 2005).

In vitro culture studies show that VEGF stimulates the maturation of bovine oocytes during IVM, resulting in increased rates of fertilization and embryonic development (Luo *et al*., 2002; Bokal *et al*., 2005). In NOW, decreased FF and serum VEGF levels are related to improved ovarian response, consequently increasing the number of oocytes retrieved, and improving the rates of fertilization and pregnancy; the reverse has also been shown, as elevated FF VEGF levels are associated with poor oocyte quality and decreased fertilization and pregnancy rates, especially in older patients (Battaglia *et al*., 2000a, b; Ocal *et al*., 2004; Artini *et al*., 2006, 2007). In women with PCOS, elevated FF VEGF is closely associated with the development of ovarian hyperstimulation syndrome (Agrawal *et al*., 1998, 2002; Artini *et al*., 1998; Franks *et al*., 2002). Furthermore, it is well known that increased FF VEGF levels in PCOS patients is indicative of immature oocytes and poor fertilization rates (Artini *et al*., 2006, 2009).

An opposing study concluded that follicles containing higher FF VEGF concentrations provide better MII oocytes, compared with those with lower FF VEGF concentrations (Bokal *et al*., 2004). Among PCOS groups, reports suggest that prolonged hCG action results in elevated FF VEGF, consequently increasing the number of high-quality oocytes and embryos, as well as improving fertilization rates (Bokal *et al*., 2005): the same researchers also demonstrated that decreases in FF VEGF and E_2 levels in PCOS women following GnRH antagonist administration have detrimental effects on follicular development, as compared with those women who were given agonists, consequently reducing oocyte and embryo quality (Bokal *et al*., 2009). Therefore, FF VEGF may serve as a dynamic indicator for the evaluation of follicular maturity, subsequently predicting oocyte maturity, fertilization success and embryo development in PCOS patients (Bokal *et al*., 2005, 2009); however, further research is required to uncover the true relationship between VEGF levels and subsequent success in PCOS women.

Cytokine family

Cytokines encompass a large family of soluble polypeptide regulators that are produced widely throughout the body by cells of diverse embryological origin; the family comprises the interleukins (IL1 ~ 35), leukemia inhibitory factor, tumor necrosis factor (TNF)α, soluble Fas (sFas) and sFas ligand (sFasL) (TNFsubfamily). Within the ovary, the action of cytokines may be autocrine or paracrine, but not endocrine; they exist in the FF, suggesting their production by GCs (Buyalos *et al*., 1992; Zolti *et al*., 1992; Jasper and Norman, 1995; Amato *et al*., 2003; Gallinelli *et al*., 2003), and have regulatory functions in follicular maturation and subsequent embryonic development (Coskun *et al*., 1998; Hsieh *et al*., 2005). In PCOS patients cytokines are believed to play a role in ovarian hyperstimulation (Pellicer *et al*., 1999) and hyperandrogenism (Escobar-Morreale *et al*., 2001); however, these reports have been disputed (Gonzalez *et al*., 1999; Deshpande *et al*., 2000; Amato *et al*., 2003).

Interleukins

ILs are a group of cytokines (secreted proteins/signaling molecules) that are expressed by leukocytes (Wu *et al*., 2007a, b). Studies have elucidated that ILs, namely IL-1, IL-2, IL-6, IL-8, IL-11, IL-12 and other cytokines, play multiple roles in folliculogenesis, ovulation and corpus luteum function (Barak *et al*., 1992; Naz and Butler, 1996; Branisteanu *et al*., 1997; Gallinelli *et al*., 2003). FF IL-12 levels vary within immature and pre-ovulatory follicles (Coskun *et al*., 1998); the presence of FF IL-12 has been associated with fertilization failure (Gazvani *et al*., 2000). An important study has demonstrated that decreased FF IL-12 level and increased FF IL-13 level in PCOS patients is correlated with a reduced rate of oocyte maturation, fertilization and pregnancy, but this reduction did not reach statistical significance (Gallinelli *et al*., 2003).

Tumor necrosis factorα

TNFα is a multifunctional hormone-like polypeptide, which is involved in a wide range of physiological roles in regulating ovarian function, exerting an influence on proliferation, differentiation, follicular maturation, steroidogenesis and apoptosis (Lédée-Bataille *et al*., 2001; van der Spuy and Dyer, 2004; Artini *et al*., 2007). In the ovary, TNFα is expressed by the oocyte, theca cells, GCs and corpora lutea (Artini *et al*., 2007). One IVM model, coupling porcine oocyte co-incubation with high levels of TNFα, reported decreased oocyte maturation and increased proportions of oocytes with abnormal chromosomal alignment and cytoskeleton structure (Ma *et al*., 2010). Alterations in FF TNFα levels are correlated with poor-quality oocytes in women undergoing IVF (Cianci *et al*., 1996; Carlberg *et al*., 2000; Lee *et al*., 2000), resulting in reduced rates of fertilization, embryonic development and pregnancy outcome (Ma *et al*., 2010). Furthermore, increased levels of FF TNFα in women with PCOS are significantly and inversely correlated to FF E_2 levels, which is again indicative of poor-quality oocytes and embryos (Gallinelli *et al*., 2003; Amato *et al*., 2003; Wu *et al*., 2007a, b; Kim *et al*., 2009).

Soluble Fas and sFas ligand

sFas and sFasL are transmembrane proteins belonging to the TNF subfamily; sFas and sFasL proteins exert anti- and pro-apoptotic functions, respectively. The binding of sFasL with its receptor induces apoptosis, whereas sFas, acting as a functional antagonist, binds with sFasL to inhibit sFasL-mediated apoptosis by preventing death signal transduction (Ueno *et al*., 1999; Onalan *et al*., 2005). sFas can be detected in human sera, oviduct fluid and FF (Srivastava *et al*., 1998; Onalan *et al*., 2005, 2006) and sFas levels in the FF are positively correlated to oocyte maturity and survival in IVF patients (Sarandakou *et al*., 2003). Some studies have demonstrated that the sFas–sFasL system involves apoptosis of theca cells and GCs in PCOS patients (Cataldo *et al*., 2000; Webber *et al*., 2003; Onalan *et al*., 2005). Furthermore, these reports suggest that reduced serum levels of sFas and DNA fragmentation in luteinized GC are found in women with PCOS undergoing IVF treatment. Patients with PCOS who are treated with metformin display anti-apoptotic effects owing to elevated serum sFas levels and reduced FF sFasL levels; GC DNA fragmentation was also reduced, thus increasing implantation and clinical pregnancy rates (Onalan *et al*., 2005). According to these data, one may speculate that abnormalities in the sFas–sFasL system are indicative of PCOS pathogenesis, further associating decreased oocyte quality, lower fertilization rates and higher miscarriage rates with PCOS.

Other microenvironment factors

Homocysteine

Homocysteine (Hcy) is a homologue of the amino acid cysteine, differing by an additional methylene group, and can be recycled into methionine or converted into cysteine in the presence of B-vitamins. Many studies have established that elevated Hcy levels in serum and FF are inversely associated with oocyte and embryo quality (Steegers-Theunissen *et al*., 1992; Ebisch *et al*., 2006; Berker *et al*., 2009; Nafiye *et al*., 2010), resulting in decreased fertilization and pregnancy rates, and increased miscarriage rates in PCOS patients undergoing IVF treatment (Ludwig *et al*., 1999; Plachot *et al*., 2003; Yarali *et al*., 2001; Loverro *et al*., 2002; Schacter *et al*., 2003; Heijnen *et al*., 2006; Kaya *et al*., 2009; Berker *et al*., 2009; Nafiye *et al*., 2010). Previous studies demonstrated that IVF patients who have higher E_2 levels in FF have improved rates of oocyte fertilization, cleavage and implantation (Botero-Ruiz *et al*., 1984; Foong *et al*., 2005; Berker *et al*., 2009). Furthermore, elevated levels of Hcy in FF and serum may suppress E_2 synthesis, and consequently interfere with ovarian follicular developmental competence, oocyte maturation and fertilization in women with PCOS (Boxmeer *et al*., 2008; Berker *et al*., 2009). Therefore, all of these results suggest that high levels of FF Hcy have a detrimental effect on oocyte and embryo quality, and may serve as a useful indicator for potential success in PCOS patients undergoing assisted reproduction.

Leptin

Leptin is a 16 kDa protein hormone that plays a key role in regulating energy intake, energy expenditure and a balance between the two. It has also served as a biomarker for body fat. In the field of assisted reproduction, leptin has been used to predict oocyte maturity and embryo quality (Barroso *et al*., 1999; Georgios *et al*., 2005). High leptin levels in the FF and serum are closely associated with decreased oocyte maturity, poor fertilization and embryo quality, and lower pregnancy rates in PCOS patients (Mantzoros *et al*., 2000; Georgios *et al*., 2005; Li *et al*., 2007). Some studies show that elevated leptin levels in women with PCOS play an elementary role in the pathogenesis of PCOS (Scarpace *et al*., 2000; Pasquali *et al*., 2006; Cervero *et al*., 2006; Li *et al*., 2007). Others suggest that elevated leptin levels in the ovary may block E_2 production, disturbing follicular development and oocyte maturation (Mantzoros *et al*., 2000). Hyperleptinemia, or increased FF leptin, in PCOS patients may impair embryo quality and pregnancy rates (Anifandis *et al*., 2005; De Placido *et al*., 2006, Li *et al*., 2007). In contrast, other investigations have shown that FF leptin is decreased in women with PCOS and is not a useful marker for oocyte quality, fertilization or embryo development (Welt *et al*., 2003; Plati *et al*., 2009). Hence, the involvement of leptin and its significance in the establishment of PCOS pathophysiology, especially its impact on oocyte maturation competence, needs further clarification.

FF meiosis-activating sterol

FF meiosis-activating sterol (FF-MAS) is an endogenous signaling molecule and an intermediate in the cholesterol biosynthetic pathway, which is present in FF (Byskov *et al*., 1999, 2002; Bokal *et al*., 2006; Grondahl, 2008). Many IVM studies demonstrate that exposure to FF-MAS can promote nuclear and cytoplasmic maturation of the oocyte (Tsafriri and Motola, 2007) and improved fertilization and early embryonic development in humans and other mammals (Cukurcam *et al*., 2003; Bivens *et al*., 2004; Faerge *et al*., 2006; Grondahl, 2008). Interestingly, reports show that FF-MAS enhances successful IVM of oocytes retrieved from women with PCOS (Chian *et al*., 2000; Grondahl, 2008). Furthermore, a leading report suggests that the concentrations of FF-MAS significantly increase during the perio-ovulatory period, between 10–14 and 34–38 h after hCG administration; this may be related to increased numbers of MII stage oocytes retrieved from PCOS patients (Bokal *et al*., 2006). This knowledge may prove to be useful in the implementation of IVM protocols for PCOS patients.

Immunoreactive corticotrophin-releasing hormone, tissue inhibitor of metalloproteinase-1 & 2 and visfatin

Immunoreactive corticotrophin-releasing hormone (IrCRH) is a 41-amino acid neuropetide (Vales *et al*., 1981), synthesized by theca cells and/or the mature oocyte itself (Mastorakos *et al*., 1993, 1994). Study has found that decreased FF IrCRH levels are correlated with oocyte dysfunction in women with PCOS (Mastorakos *et al*., 1994). Other reports suggest that FF tissue inhibitor of metalloproteinase (TIMP)-1 & 2 levels are significantly lower in women with PCOS than in NOW (Lahav-Bratz *et al*., 2003). In contrast, there was no difference in basal production of TIMP-1 by cells in culture between women with PCOS and NOW; however, matrix metalloproteinases-2 and 9 are significantly increased in the FF of women with PCOS (Shalev *et al*., 2001), suggesting an association with inappropriate atresia. In a recent study, serum visfatin levels were significantly increased in women with PCOS, whereas FF visfatin levels do not differ when compared with non-PCOS patients (Plati *et al*., 2009). On the basis of these studies, it is difficult to assign specific effects to these factors, although their association to physiological or pathological functions in PCOS is evident.

Renin

Renin (also known as angiotensinogenase) participates in the body's renin–angiotensin system. It is known that ovarian renin has an impact on the developmental and fertilization competence of human oocytes (Itskovitz *et al*., 1991; Van Blerkom *et al*., 1997; Loret de Mola *et al*., 1999; Bokal *et al*., 2005). Investigations suggest that decreased FF renin is related to increased rates of oocyte maturation and fertilization, and better subsequent embryo quality (Bokal *et al*., 2003, 2004, 2005).

Resistin

Resistin is a 12.5 kDa cyteine-rich protein hormone, synthesized by adipose tissues (Seow *et al*., 2005). Recent studies demonstrate that there are no significant differences in either serum or FF resistin concentrations between PCOS patients and controls; these are also not significantly correlated with fertilization rates, implantation rates, clinical pregnancy rates or early miscarriage rates in PCOS patients (Seow *et al*., 2005). These data indicate that resistin is unlikely to be a useful biomarker for oocyte developmental competence during IVF treatment in PCOS women.

Oxidative stress

Reactive oxygen species (ROS) are involved in many physiological functions and act as mediators in a variety of signaling pathways.

Damage to biological systems caused by an excess of ROS is referred to as OS (Gupta *et al*., 2009). In women with PCOS, data show that increased FF ROS and decreased total antioxidant capacity and superoxide dismutase are closely associated with lower rates of oocyte maturation and fertilization, poor embryo quality and decreased pregnancy rates (Sabatini *et al*., 2000; Ruder *et al*., 2008; Bausenwein *et al*., 2010; Chattopadhayay *et al*., 2010). ROS degrade polyunsaturated lipids, forming malondialdehyde (MDA) (Pryor and Stanley, 1975). Elevated FF MDA levels are directly correlated with increased numbers of immature oocytes retrieved, lower rates of fertilization and embryonic development, and consequently, lower pregnancy rates in PCOS patients (Yildirim *et al*., 2007; Berker *et al*., 2009). Therefore, ROS may impair oocyte quality via alterations in the balance of FFFs in the follicular microenvironment.

Concluding remarks

Patients with PCOS are typically characterized by production of an increased numbers of oocytes during stimulation in an IVF cycle; however, these women suffer from poor-quality oocytes and embryos, lower fertilization, cleavage and implantation rates, and higher miscarriage rates. A series of extra- and intra-ovarian factors causing abnormalities during folliculogeneis, follicular growth and oocyte meiotic maturation processes have been identified. Whether these abnormalities have a direct influence on GC–oocyte interactions and oocyte meiotic maturation, fertilization, embryonic development and pregnancy, or whether the influences are through circulating endocrine and local paracrine/autocrine mechanisms, requires further clarification. Although many studies have been performed in all aspects of endocrinology, genetics, metabolism and reproduction in the etiology and pathology of PCOS, it remains a challenge for clinical and academic scientists alike to elucidate the molecular mechanisms involved; in particular, the oocyte's developmental competence and genetic disruption are undoubtedly important considerations. Therefore, systematic screening for key intra-ovarian factors which are related to PCOS (such as AMH, Hcy, growth factors and cytokines) coupled with proper treatment for each PCOS phenotype are essential issues in achieving success for PCOS patients undergoing assisted reproduction, in an effort to effectively improve oocyte maturation and developmental competence.

Authors' roles

J.Q. is responsible for data collection and outline design; H.L.F. is responsible for manuscript preparation.

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