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Implications of Polycystic Ovary Syndrome (PCOS) on Oocyte Development

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Abstract

Human follicle development requires the recruitment of primordial follicles into a cohort of growing follicles from which one follicle is selected to ovulate a mature oocyte. During this developmental process, complex endocrine and intraovarian paracrine signals create a changing intrafollicular hormonal mileu. With this microenvironment, appropriate cumulus cell-oocyte signaling governs oocyte developmental competence, defined as the ability of the oocyte to complete meiosis and undergo fertilization, embryogenesis and term development. Many of these mechanisms are perturbed in polycystic ovary syndrome (PCOS), a heterogeneous syndrome characterized by ovarian hyperandrogenism, hyperinsulinemia from insulin resistance and reduced fecundity. In addition to these endocrinopathies, PCOS also is characterized by paracrine dysregulation of follicle development by intraovarian proteins of the transforming growth factor β (TGFβ) family. Consequently, PCOS patients undergoing ovarian stimulation for *in vitro* fertilization (IVF) are at increased risks of impaired oocyte developmental competence, implantation failure and pregnancy loss. Recent data demonstrate links between endocrine/paracrine factors and oocyte gene expression in PCOS and suggest that new clinical strategies to optimize developmental competence of PCOS oocytes should target correction of the entire follicle growth and oocyte development process.

Keywords

Polycystic ovary syndrome; follicle development; hyperandrogenism; hyperinsulinemia; oocyte maturation

Introduction

Human follicle development is an ordered process, in which primordial follicles are recruited into a cohort of growing follicles, from which one follicle is selected to ovulate a mature oocyte. Theca cell-granulosa cell interactions, intraovarian paracrine signals and oocyte secreted factors control preantral and early antral follicle development, and are regulated by several factors [1], including transforming growth factor β (TGFβ)-related proteins [2-4]. Circulating gonadotropins and intraovarian paracrine signals govern subsequent antral follicle development. Throughout folliculogenesis, a changing intrafollicular microenvironment established by various proteins, steroids, energy metabolites, cytokines and growth factors [5]

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appropriately coordinates follicle growth and oocyte development [6]. Within this intrafollicular microenvironment, appropriate cumulus cell-oocyte signaling induces the acquisition of oocyte developmental competence, defined as the ability of the oocyte to complete meiosis and undergo fertilization, embryogenesis and term development [7].

Many of these mechanisms are perturbed in polycystic ovary syndrome (PCOS), a heterogeneous syndrome characterized by luteinizing hormone (LH) hypersecretion, ovarian hyperandrogenism, polycystic ovaries, hyperinsulinemia from insulin resistance and reduced fecundity [8]. PCOS patients undergoing ovarian stimulation for *in vitro* fertilization (IVF) have increased risks of impaired oocyte developmental competence, implantation failure and pregnancy loss [9-11]. Moreover, obese PCOS patients experience low oocyte fertilization and failure of embryos to implant in their own uterus or those of their surrogates, implying impaired development competence of some PCOS oocytes [12]. This chapter addresses crucial endocrine and intraovarian paracrine mechanisms governing follicular development and discusses how PCOS-related alterations of these mechanisms may impair oocyte developmental competence.

Follicular Development

The primordial follicle comprises an oocyte arrested in meiotic prophase I and surrounded by squamous granulosa cells (i.e., a germinal vesicle [GV] stage oocyte). When the primordial follicle initiates growth, its oocyte undergoes changes in messenger ribonucleic acid (mRNA) expression as its squamous granulosa cells enlarge into a single layer of cuboidal granulosa cells (i.e., primary follicle) [13, 14]. With continued granulosa cell proliferation into several layers, the secondary follicle is formed. Theca cells organize into distinct layers around the follicle and establish mesenchymal-epithelial cell interactions that promote development of the follicle and its oocyte.

Primordial follicle growth is only minimally dependent upon follicle-stimulating hormone (FSH) and is primarily influenced by paracrine/endocrine factors [14]. Granulosa cell-derived paracrine factors either can activate resting primordial follicles (e.g., kit ligand, transforming growth factor-α, epidermal growth factor) or can inhibit them (i.e., anti-mullerian hormone [AMH]), and may originate locally or from neighboring growing follicles responsive to FSH [1, 14,15]. Oocyte-derived factors (growth differentiation factor 9 [GDF9)], bone morphogenetic protein 15 [BMP15]) also control follicle growth [2-4]. These granulosa cell-oocyte interactions cause preantral follicles to develop over several months; to acquire FSH, estrogen and androgen receptors; and to become physiologically coupled by gap junctions [14, 16, 17].

Antral follicle formation is accompanied by diminished oocyte growth (reaching a maximum diameter of 140 μm), creation of extracellular fluid and differentiation of granulosa cell layers into mural and cumulus cell subpopulations [13, 14]. Human antral follicles 2-5 mm in size become responsive to FSH [16], while those 6-8 mm in size acquire aromatase activity [18], allowing androgens produced by LH-stimulated theca cells to undergo aromatization to estrogens by FSH-stimulated granulosa cells. Estrogen production is facilitated by granulosa cell-derived paracrine regulation of theca cell $P450_{c17}$ activity [19], with inhibins and insulin-like growth factor (IGF)1 stimulating aromatizable androgen production, and follistatin binding activin and inhibiting its androgen-suppressing effect [20]. With the ability of FSH to induce LH receptors on granulosa cells, allowing them to respond to both gonadotropins, the maturing follicle continues growth and steroidogenesis despite declining FSH levels in the circulation before the midcycle LH surge [16, 21].

With the onset of the midcycle LH surge, the preovulatory follicle shifts steroidogenesis from androgen and estrogen to progesterone production during final oocyte maturation [16]. In reproductive-aged women, hundreds of primordial follicles initiate growth, 10-20 selectable antral follicles remain at the beginning of the normal cycle, but just one normally proceeds to

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ovulation [13]. Follicular growth from primordial to preovulatory stage takes approximately 6 months, with the final 2 weeks of follicular development dependent upon changes in circulating gonadotropin levels [16].

Oocyte maturation

In mammals, developmentally competent oocytes have the necessary molecular components to complete meiosis, enter the mitotic cell cycle, create an embryonic genome, modify its chromatin structure, and transcribe the correct genes to begin the developmental program [22]. These processes depend upon maternal mRNAs, proteins, and other molecular components of the oocyte acquired during its development. Acquisition of oocyte developmental competence occurs progressively throughout oocyte growth and maturation [23, 24] and is closely coordinated with follicular development [6].

With recruitment of primordial follicles into the growing preantral follicle pool, mammalian GV oocytes undergo intensive mRNA synthesis [25] and other structural as well as functional changes [23, 24, 26]. Transcripts synthesized at this time are either used during oocyte growth or stored as ribonucleoproteins for later use during oocyte maturation and early preimplantation embryogenesis [22, 25]. Intense RNA transcription is completed with maximal oocyte growth [26, 27], at which time oocytes are competent to complete meiotic maturation, but have not acquired full developmental competence.

Oocytes undergo further maturation with follicle growth [28]. Transition from the GV-stage to the metaphase II-stage of mouse oocyte development is associated with transcriptional silencing and selective destruction of transcripts involving meiotic arrest, oxidative phosphorylation, energy production, protein synthesis and metabolism [29]; post-transcriptional mRNA modifications and post-translational protein alterations in several species, however, continue to metaphase II [30, 31]. Bovine oocytes exposed to increasing levels of estradiol (E2) and growth factors during follicle development also show morphological changes in lipid accumulation and nucleolar vacuolization [32]. Protein synthesis essential for embryogenesis occurs predominantly before GV breakdown in bovine oocytes [31], being further modified by steroids in maturing ovine and porcine oocytes [23, 33, 34]. Consequently, in cattle and sheep, *in vitro* matured oocytes from large antral follicles are more competent to develop into blastocysts than similarly-matured oocytes from small antral follicles [35, 36], with FSH also enhancing oocyte developmental competence in primates [37, 38]. A similar relationship between follicle size and oocyte developmental competence likely exists for *in vivo* matured oocytes in humans [39-41].

With the preovulatory LH surge, the mammalian oocyte undergoes GV breakdown (metaphase I) and produces a haploid oocyte (metaphase II), at which time transcription and protein synthesis decline to basal levels [31], and the oocyte becomes capable of fertilization and initial embryonic development. Progression of the oocyte to the first meiotic metaphase (i.e., nuclear [meiotic] maturation) occurs together with cytoplasmic maturation, which involves recruitment and post-transcriptional modifications of mRNAs, translation of dormant transcripts, and post-translational modifications of proteins essential for fertilization and embryogenesis [30,42]. Bidirectional cumulus cell-oocyte signaling via gap junctions also is essential for mammalian oocyte developmental competence [6], and may depend upon increasing progesterone levels [34].

PCOS and oocyte development

Beyond our basic understanding of oocyte physiology, there are several reasons why the relationship between PCOS and oocyte developmental competence remains unclear. First, the microenvironment of each follicle is unique and has its own effect on the developing oocyte

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[43]. Second, multiple embryos are transferred simultaneously into the uterus during IVF, confusing relationships between follicle fluid steroid levels and embryo implantation. Finally, studies of oocyte developmental competence are limited by ethical and experimental constraints on the use of human oocytes and embryos for biomedical research. Therefore the following discussion of oocyte development in PCOS is based upon indirect markers of oocyte developmental competence, which include studies of oocyte gene expression, correlations between follicle fluid steroid levels and oocyte development *in vivo*, effects of enzymatic disruption of steroidogenesis on oocyte development *in vivo*, and actions of sex steroids on oocyte development *in vitro*.

Ovarian hyperandrogenism

Androgens promote early follicle growth in primates. Testosterone administration to adult female rhesus monkeys increases the number of primary, growing preantral and small antral follicles and the proliferation of granulosa cells within them by acting through its own receptor [44, 45]. Androgen treatment in such monkeys also increases mRNA expression of FSH receptor, IGF1 receptor and IGF1 in granulosa cells [46, 47], while enhancing IGF1 and IGF1 receptor mRNA expression in primordial follicle oocytes [48].

Human preantral follicles also express mRNA for androgen receptor [17] and grow in response to androgen exposure since antral follicle numbers in women positively correlate with serum androstenedione levels [49, 50]. As a result, intrinsic ovarian hyperandrogenism in PCOS [51] is accompanied by 1) abnormal oocyte gene expression in preantral follicles (see TGFβ-related genes), 2) hyperandrogenism in small antral follicles [52] and 3) development of PCO morphology [53, 54]. The collective data strongly suggest that PCOS exerts complex effects on the oocyte that begin with early preantral follicle growth and continue during later follicle development.

Androgen also interferes with E2-dependent signaling mechanisms accompanying oocyte cytoplasmic maturation. Exposure of cultured immature human oocytes to E2 bound to albumen increases fertilization and cleavage rates of *in vitro* matured oocytes, without affecting nuclear maturation (Figure 1) [55]. Such E2 action on *in vitro* matured human oocytes is accompanied by increased oscillations of intracellular free calcium, which are antagonized by androgen [56]. Consequently E2/androgen ratios to which immature human oocytes are exposed in the follicular phase appear to affect the quality of mature human oocytes obtained through IVF. In support of this, pregnancy outcome by IVF is related more to the E2/androgen ratio than to the absolute amount of E2 in the follicle [57].

Moreover, small PCOS follicles have elevated 5α-reductase activity, which increases 5α-reduced androgens to levels capable of inhibiting granulosa cell aromatase activity *in vitro* and harming oocytes through limited E2 production [58, 59]. As an animal model of PCOS, adult female rhesus monkeys exposed to prenatal androgen excess in early gestation and later subjected to FSH therapy show increased 5α-reductase and decreased aromatase activities in E2-deficient follicles [43] accompanied by impaired blastocyst development after combined rhFSH/human chorionic gonadotropin therapy [60]. Consistent with E2-enhanced oocyte development in human and nonhuman primates [55, 61], low E2 production in IVF patients with 17α-hydroxylase deficiency is associated with *in vitro* embryonic developmental arrest [62]. Conversely, follicle fluid E2 content in IVF patients positively correlates with oocyte fertilization, cleavage and implantation [62], with E2 levels in follicles containing *in vitro* fertilized oocytes being higher in women who conceive versus those who do not after embryo transfer [57, 62].

In PCOS patients undergoing ovarian stimulation for IVF, terminally differentiated follicles remain hyperandogenic [63] and contain meiotically-competent (metaphase II) oocytes with

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distinctly abnormal gene expression profiles (Figure 2) [64]. Many of these differentially expressed genes in PCOS involve signal transduction, transcription, deoxyribonucleic acid and RNA processing and the cell cycle. Many of them also share promoter sequences containing putative transcription factor binding sites with sequence homology for androgen receptor, peroxisome proliferating receptor gamma and/or peroxisome proliferating receptor gamma-retinoid X receptor binding sites [64]. These findings combined with androgen receptor [65] and insulin receptor mRNA expression [66] in human cumulus and mural granulosa cells provide rational physiological mechanisms by which endocrine factors can normally regulate appropriate follicle growth and oocyte development. Moreover, such cumulus and mural granulosa cell receptor expression also provides the physiological bases by which PCOS-related hyperandrogenism or adiposity-dependent insulin resistance perturbs cumulus-oocyte signaling.

Hyperinsulinemia and premature follicle luteinization

Insulin binds to its own receptors located on theca cells, surrounding stroma, granulosa cells and oocytes [66, 67] to promote follicle recruitment [68] and to stimulate theca cell [69] as well as granulosa cell steroidogenesis [70]. Insulin stimulates theca cell androgen production by stimulating 17a-hydroxylase activity, amplifying LH- and IGF1-stimulated androgen production, elevating serum free testosterone levels through decreased hepatic sex hormone-binding globulin production, and enhancing serum IGF1 bioactivity through suppressed IGF-binding protein production [69]. Insulin also enhances FSH-induced upregulation of LH receptors in granulosa cells and increases their ability to produce P4 in response to LH [71, 72].

Insulin sensitivity in PCOS patients is intrinsically impaired from abnormal post-receptor signal transduction, reducing insulin-mediated glucose uptake without affecting steroidogenesis [69]. As a result, PCOS patients have insulin resistance independent and additive with that of obesity, with combined PCOS and obesity synergistically impairing glucose-insulin homeostasis and promoting ovarian steroidogenesis. This is presumably why hyperinsulinemia from insulin resistance in PCOS enhances androgen production [69] and also induces premature granulosa cell luteinization [70, 71], leading to arrest of cell proliferation and follicle growth. Consequently, small antral PCOS follicles exhibit P4 hypersecretion and overexpress LH receptors [21, 73] and also show an exaggerated shift in steroidogenesis from E2 to progesterone production [71]. A comparable steroidogenic shift occurs in early-treated prenatally androgenized adult female rhesus monkeys undergoing gonadotropin therapy for IVF, in which combined LH hypersecretion and relative insulin excess at oocyte retrieval is accompanied by impaired blastocyst development [60]. The further observation that insulin together with FSH upregulates LH receptor expression in cultured mouse cumulus-oocyte complexes and reduces blastocyst development [72] provides additional evidence that insulin excess might perturb oocyte development through altered cumulus-oocyte signaling.

Consistent with this hypothesis, the amount of insulin present in the human follicle is determined by BMI and fasting serum insulin levels, and is highest in women with impaired glucose tolerance (Figure 3) [66]. Therefore, the use of the metformin to improve insulin sensitivity has been proposed as a strategy to improve follicular growth and oocyte development in PCOS. In one of two prospective, randomized, double blind studies, pretreatment of PCOS patients with metformin preceding GnRH analog/rhFSH therapy for IVF did not affect ovarian responsiveness to FSH therapy nor pregnancy outcome [74]. In the other, metformin therapy to PCOS women lowered serum fasting insulin, total and free testosterone as well as E2 levels at oocyte retrieval, enhanced clinical pregnancy and livebirth rates, and diminished the risk of severe ovarian hyperstimulation syndrome [75]. In a recent double-blind, randomized study powered to examine live birth rate, however, metformin lacked

superiority over clomiphene citrate in achieving live-birth in 626 infertile PCOS patients, making the current use of metformin to improve developmental competence of PCOS oocytes controversial[76].

TGFβ-related proteins

Follicle growth and oocyte development are regulated by several proteins of the TGFβ family, including activins, inhibins, AMH, GDF9 and BMP15 [77]. Many of these factors are produced by the oocyte (i.e., GDF9 and BMP15) and its surrounding granulosa cells (i.e., activins, inhibins, AMH) and interact with each other to coordinate granulosa cell-oocyte signaling. As an oocyte-secreted factor, GDF9 promotes granulosa cell proliferation and preantral growth [78-80], while its deficiency in mice impairs granulosa cell proliferation and causes follicular arrest at the primary follicle stage [78]. In humans, GDF9 induces the growth of human ovarian follicles *in vitro* [81]. Oocyte GDF9 expression normally begins in humans at the primordial-primary follicle transition and increases with preantral follicle growth [82, 83]. Reduced *GDF9* mRNA levels in PCOS oocytes from initiation of primordial follicle growth through the small antral follicle stage of development accompanies impaired follicle growth, presumably from altered granulosa cell-oocyte signaling (Figure 4) [54, 83].

As another TGFβ family member, AMH is normally produced by granulosa cells of growing follicles [77, 84]. Low AMH levels occur in primordial and primary follicles, increase to maximal levels in large preantral and small antral stages, and then decline during final follicular maturation [84-87]. *In vitro* rodent studies show that AMH inhibits primordial follicle growth [15], while its deficiency has the opposite effect [88], suggesting that AMH produced by growing follicles inhibits growth of adjacent primordial follicles [84, 89]. Histological examination of human ovaries shows reduced AMH levels in primordial and transitional follicles of PCOS patients, implicating relative AMH deficiency as an additional factor involved with abnormal growth of the primordial follicle and its oocyte (Figure 5) [84].

Granulosa cell-derived inhibins and activins are dimeric glycoproteins. Inhibins consist of an α-subunit covalently joined by disulfide links to either a βA-subunit (inhibin A) or a βB-subunit (inhibin B) and suppress FSH synthesis. Dimerization of β subunits produces 3 forms of activin (activin A [βA-βA], activin AB βA-βB] and activin B [βB-βB]) that enhance FSH secretion [90]. Follistatin, a glycoprotein structurally unrelated to the TGFβ superfamily, binds activin to inhibit its action [90]. Collectively, activins promote follicular development by enhancing granulosa cell responsiveness to FSH, suppressing androgen synthesis and stimulating oocyte maturation, while inhibins produced by the dominant follicle stimulate theca cell androgen production for E2 synthesis [90, 91]. In some PCOS patients, low serum activin A and high serum follistatin levels are observed [92, 93], while in others the normal intrafollicular shift from an activin-dominant to an inhibin-dominant microenvironment during follicle growth [94] is impaired [95, 96]. The clinical implications of abnormal intraovarian activin and inhibin production from PCOS on oocyte development remain uncertain.

Conclusion

Polycystic ovary syndrome is characterized by ovarian hyperandrogenism, hyperinsulinemia from insulin resistance and paracrine dysregulation of several TGFβ-related proteins, all of which can perturb the intrafollicular environment. Acting directly or indirectly through cumulus cell-oocyte signaling, the abnormal intrafollicular environment induced by PCOS has the capacity to perturb cytoplasmic and/or nuclear maturation of the oocyte and to alter oocyte gene expression important for embryonic gene activation. Understanding how endocrine/paracrine factors and genes interact to promote oocyte developmental competence may provide

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new clinical strategies that target long-term correction of follicle growth and oocyte development.

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Abbreviations

PCOS, polycystic ovary syndrome
TGFβ, transforming growth factorβ
IVF, *in vitro* fertilization
LH, luteinizing hormone
GV, germinal vesicle
mRNA, messenger ribonucleic acid
FSH, follicle-stimulating hormone
GDF9, growth differentiation factor 9
BMP15, bone morphogenetic protein 15
IGF, insulin-like growth factor
E2, estradiol
AMH, anti-mullerian hormone
PCA, principal component analysis

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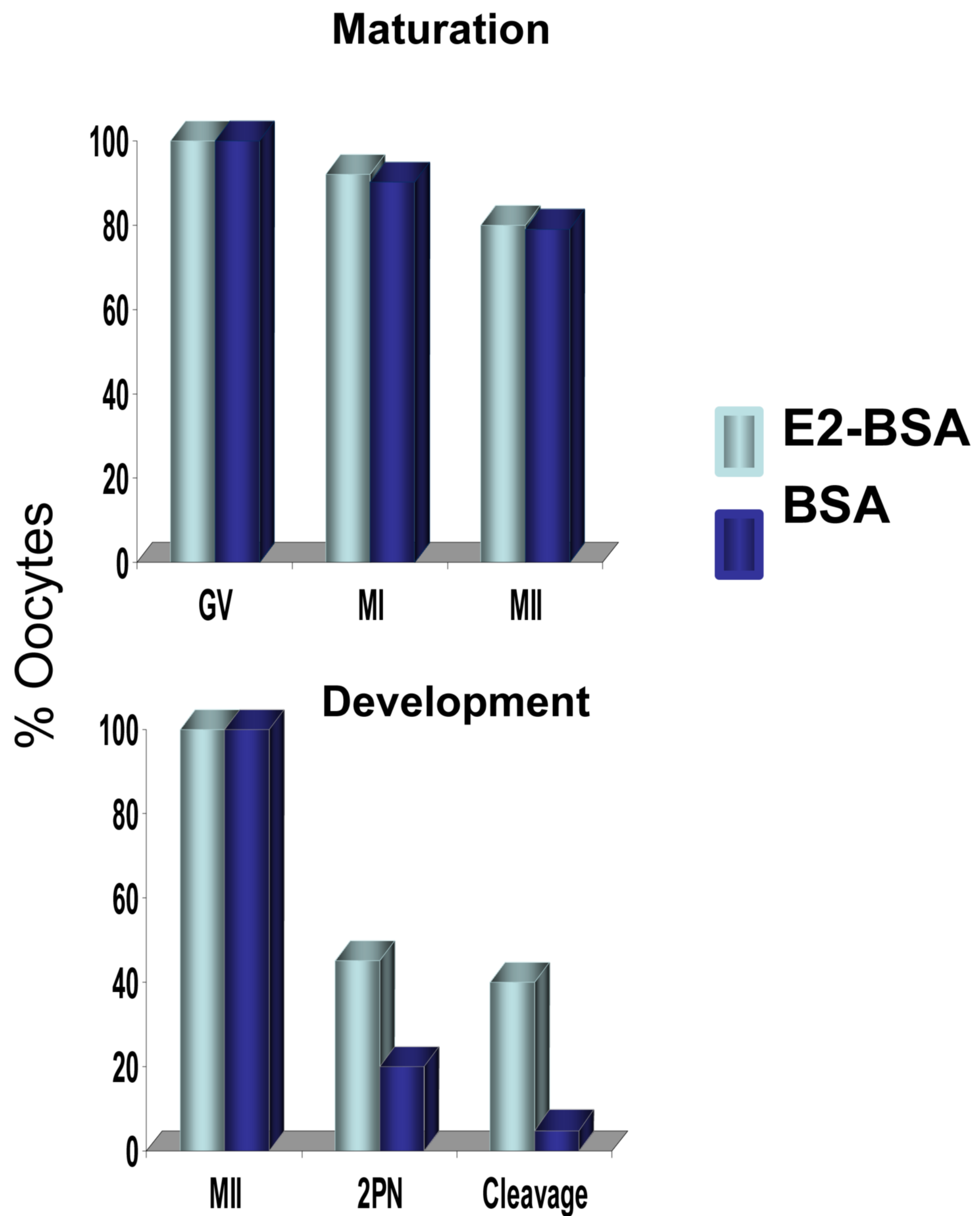

Figure 1.

Human oocytes matured *in vitro* with and without E2. (With permission: Tesarik J, Mendoza C. Nongenomic effects of 17β-estradiol on maturing human oocytes: relationship to oocyte developmental potential. J Clin Endocrinol Metab 1995;80:1438-1443)

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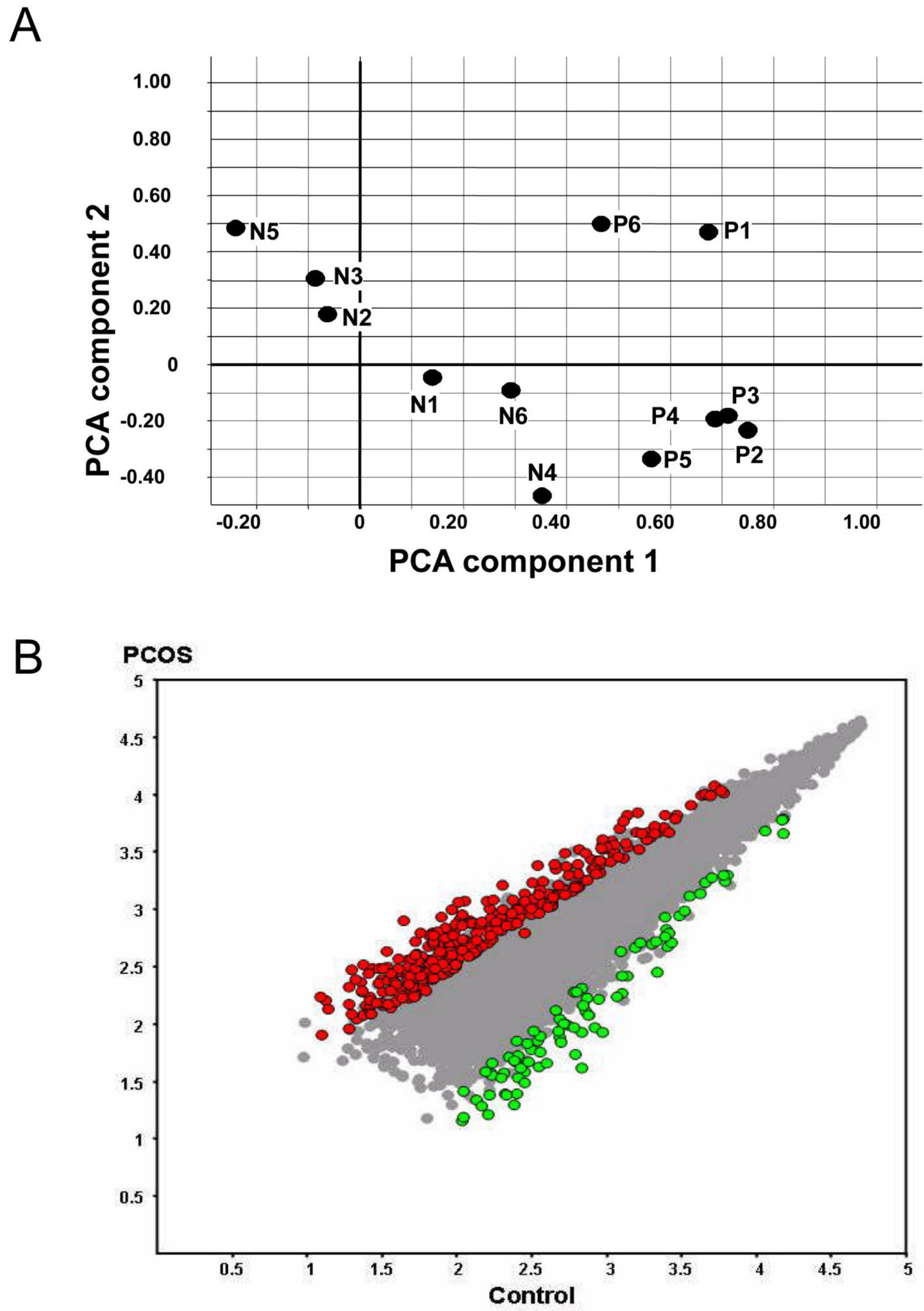

Figure 2.

Gene expression profiles of 6 normal (N1-N6) and PCOS (P1-P6) oocytes. A) Principal Component Analysis, B) Differentially expressed mRNAs in PCOS vs. normal oocytes. (With permission: Wood JR, Dumesic DA, Abbott DH, et al. Molecular abnormalities in oocytes from women with polycystic ovary syndrome revealed by microarray analysis. J Clin Endocrinol Metab 2007;92:705-713)

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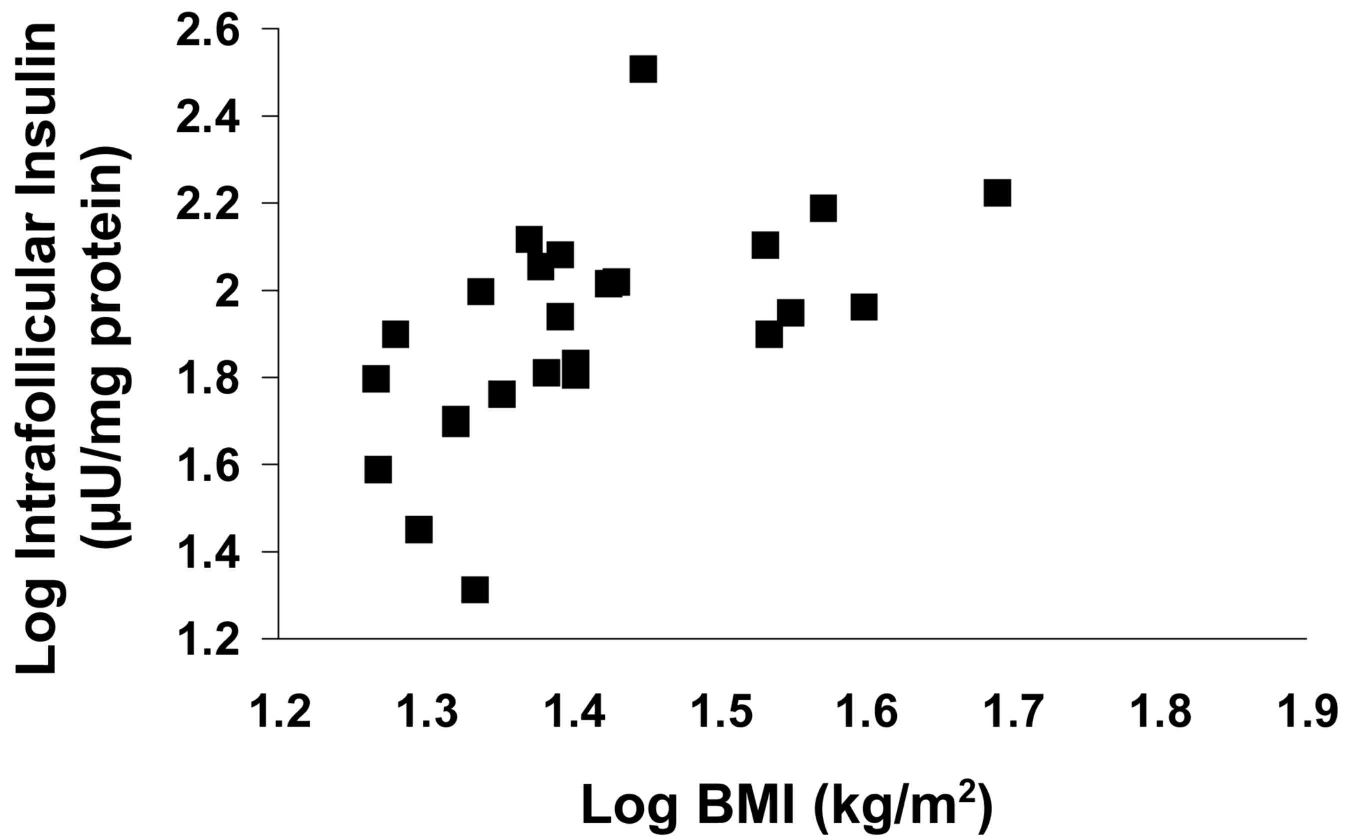

Figure 3.
Correlation between intrafollicular insulin levels and BMI in women undergoing ovarian stimulation for IVF. (With permission: Dumesic DA, Schramm RD, Abbott DH. Early Origins of Polycystic Ovary Syndrome (PCOS). Reprod Fertil Dev 2005;17:349-360)

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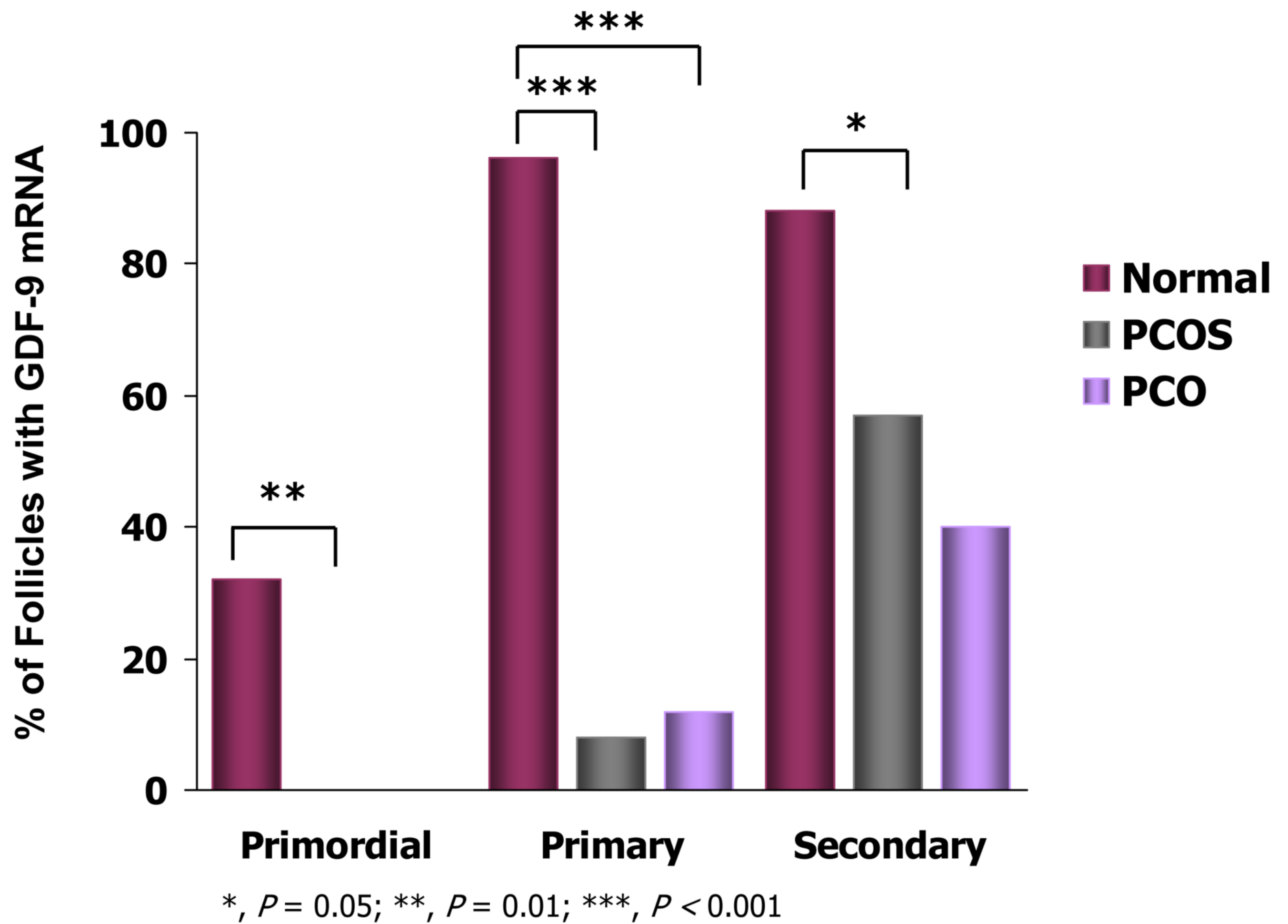

Figure 4.
Percent human preantral follicles with *GDF9* mRNA Expression.. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$. (With permission: Filho FLT, Baracat EC, Lee TH, et al. Aberrant expression of growth differentiation factor-9 in oocytes of women with polycystic ovary syndrome. J Clin Endocrinol Metab 2002;87:1337-1344)

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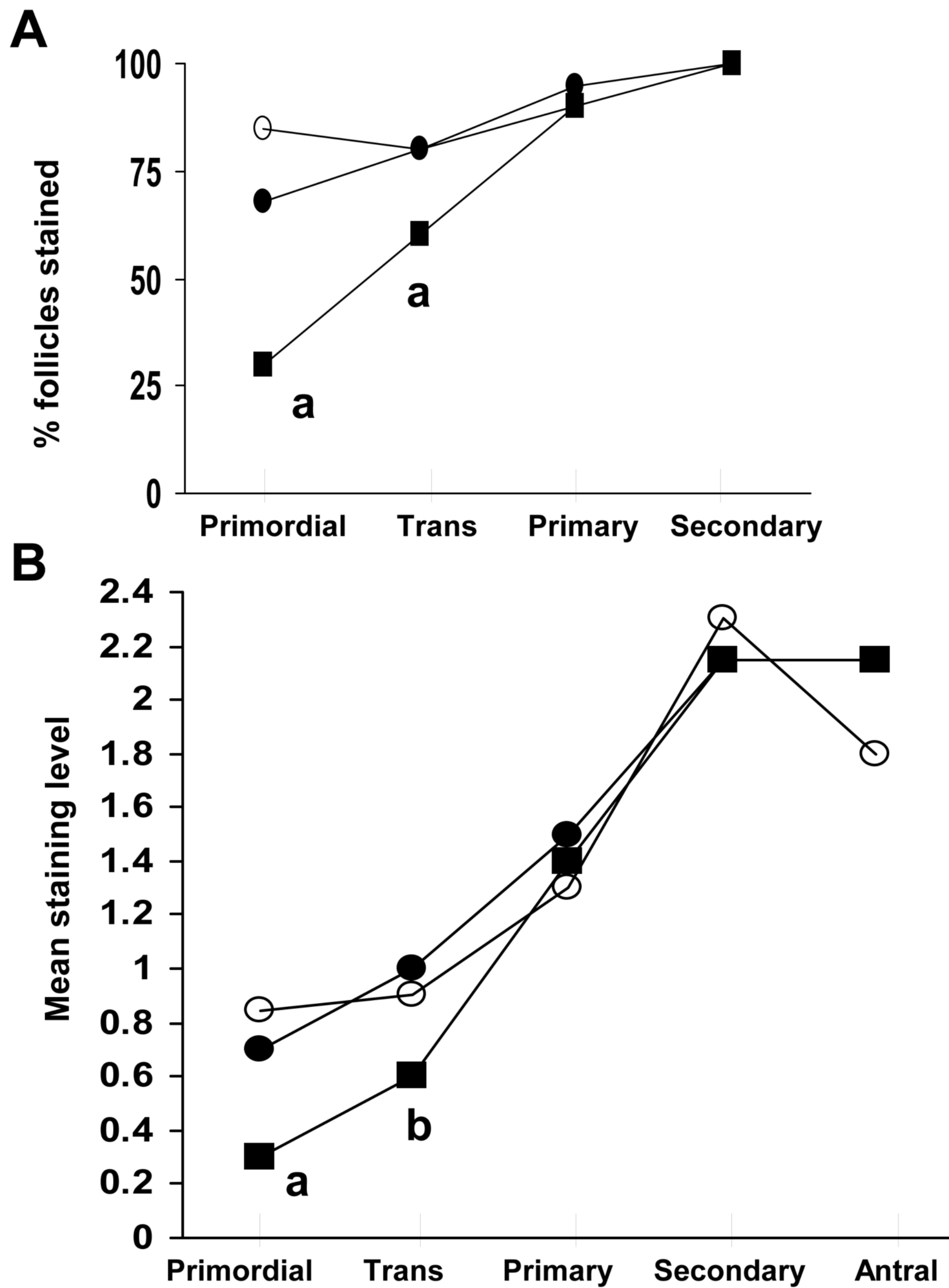

Figure 5.

A) Percent human follicles with AMH staining and B) mean intensity of AMH staining. a, *P*<0.005 vs. normal ovaries; b, *P*<0.005 vs. ovulatory PCO ovaries; c, *P*<0.005, d, *P*<0.05 vs. normal and ovulatory PCO ovaries. (With permission: Stubbs SA, Hardy K, Da Silva-Buttkus P, et al. Anti-mullerian hormone protein expression is reduced during the initial stages of follicle development in human polycystic ovaries. J Clin Endocrinol Metab 2005;90:5536-5543 Copyright 2005, The Endocrine Society)