ART failure: oocyte contributions to unsuccessful fertilization

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BACKGROUND: The complexity of fertilization failure during assisted reproductive technologies (ART) is often under-appreciated, as this failure can occur at any number of essential mechanistic and cellular events. Importantly, successful fertilization is heavily dependent upon inherent qualities of the oocytes, and thus reliant upon fidelity of oocyte maturation. METHODS: Pubmed and medline were searched up to April 2008 for papers on oocyte fertilization and its mechanistic components. References to clinical/human studies were selected wherever possible. RESULTS: Successful oocyte maturation cannot simply be determined via visual assessment of polar body extrusion, but rather entails coordination of numerous cytoplasmic processes not readily observed. Proper regulation of intra-oocyte signaling cascades is crucial for sufficient production and storage of carbohydrates and proteins, successful relocation of organelles and regulation of metabolic pathways required for an apparently mature metaphase II oocyte to complete subsequent fertilization events; such as cumulus penetration, sperm/oocyte binding, fusion, oocyte activation, sperm processing and pronuclear (PN) formation. Regulation of oocyte maturation begins during oocyte growth and is intimately connected with events influencing folliculogenesis. Therefore, the oocyte is subject to a multitude of potential effector impacting fertilization potential and developmental competence long before encountering the artificial environment of the IVF laboratory. CONCLUSIONS: Although meticulous care and continued research is essential for future improvement, failure to fertilize and properly form PN following clinical ART is likely to be dependent on historical events in oocyte maturation, not easily explained or prevented through simple modification of contemporary laboratory protocols.

Keywords: fertilization failure; oocyte maturation; oocyte quality; ART failure; IVF failure

Introduction

It is not uncommon in this age of scientific achievement, through the use of sophisticated facilities and modern technology, for the contemporary IVF laboratory to achieve fertilization rates approaching 70–80%. This is a tremendous accomplishment and an immense improvement compared to rates obtained just a few short years ago. However, extremely troublesome and upsetting is the still all-too-familiar scenario involving a case with total fertilization failure, despite the apparent lack of predictors or visual indicators. This unfortunate occurrence leaves the clinician and embryologist perplexed, wondering ‘What went wrong?’, often inferring that the error occurred sometime within the previous 15–18 h time period. When several oocytes are retrieved, failure to form the appropriate number of pronuclei (PN) in a few of them may be an acceptable loss. However, as in the catastrophic scenario above, or when few oocytes are retrieved, failure is problematic. Frustration increases when oocytes failing to fertilize appeared to be healthy displaying a single polar body, ‘normal-looking’ cytoplasm, appropriate zona thickness and proper perivitelline spacing. To combat failed fertilization, many clinics tend to err on the side of caution and subject oocytes to intracytoplasmic sperm injection (ICSI). However, although this procedure has its applications, as in cases of male factor infertility, failure still occurs in many apparently mature oocytes despite these presumably preemptive precautionary measures. In fact, following ICSI, human oocytes still fail to fertilize ~30% of the time (Payne et al., 1994; Flaherty et al., 1995), and complete fertilization failure occurs at an estimated rate of 2–3% (Mahutte and Arici, 2003). Thus, it is clear that ICSI addresses only one of the necessary components required for successful fertilization, sperm penetration. A multitude of post-sperm penetration processes must also occur for successful fertilization. In reality, inherent qualities of the oocyte are largely responsible for regulating the majority of molecular and cellular mechanisms required for fertilization events. This fact is extremely evident when one considers the occurrence of parthenogenesis, where the oocyte alone is directing a select portion of fertilization events. Therefore, factors affecting oocyte growth and development, such as the underlying pathophysiology of infertility, the management of ovulation induction and the perpetual process of atresia beginning at the 20th week in utero, all have profound influences on fertilization. These factors suggest that a portion of retrieved oocytes are likely to be inherently compromised, and thus, despite our best
efforts, destined for fertilization failure. Furthermore, it is highly unlikely that a single effector or explanation can adequately account for failure to form PN. That being said, to begin to understand why fertilization failure occurs, elucidating regulatory mechanisms involved in oocyte growth and maturation is imperative.

The purpose of this review is to update practitioners of assisted reproductive technologies (ART) on the processes of oocyte maturation, highlighting the importance of oocyte-controlled events for successful fertilization. This will include major mechanistic events involved in transition of a metaphase II (MII) oocyte to a PN stage zygote, focusing on how improper or inadequate oocyte growth and maturation can adversely affect these fertilization events. Together, this information should make it readily apparent that simple morphologic assessment, both before and after exposure to spermatozoa, is not an adequate measure of oocyte competence; just as the sole act of penetration of the oocyte by sperm in no way signifies successful fertilization.

**Materials and Methods**

Pubmed and medline were searched up to the end of April 2008 using keywords oocytes, fertilization and derivatives therein. Additional searches were made using keywords for mechanistic events, e.g. cumulus penetration, fusion. References were selected which related to clinical/human work whenever possible.

**Oocyte maturation**

Indeed, proper completion of fertilization events is deeply rooted in proper oocyte maturation. Simply defined, oocyte maturation refers to the completion of the first meiotic division and accompanying processes essential for subsequent fertilization and embryo development, and consists of two general components: nuclear and cytoplasmic maturation.

**Nuclear maturation**

At the time of birth, mammalian oocytes are arrested in the diplo-tene stage of prophase of meiosis I and remain arrested in this state while completing their growth. This stage is characterized by the presence of an intact nuclear envelope (NE) or germinal vesicle (GV). A surge of gonadotrophins preceding ovulation releases fully-grown oocytes from a quiescent state and signals re-initiation of meiosis. This process is characterized, in part, by dissolution of the GV in a process known as GV-breakdown (GVBD). During completion of prophase of the first meiotic division, homologous chromosomes undergo a process of pairing and recombination. Homologues then condense in preparation for a reductional division, resulting in disproportionate cytokinesis and extrusion of half their genetic material within the first polar body. Finally, oocytes proceed directly to MII, forgoing interphase and DNA duplication, where they are referred to as secondary oocytes, and remain until fertilization occurs (see Fig. 1).

From this description, it is evident only two characteristics of successful oocyte nuclear maturation can be visually assessed at the light microscope level; GVBD and extrusion of the first polar body. However, critical components of oocyte nuclear maturation essential for successful fertilization, such as proper condensation and alignment of chromosomes, accurate spindle formation/function, and fidelity of chromosome separation and segregation cannot be discerned. Thus, simply assuming oocytes are healthy and competent to fertilize based upon presence of the first polar body may not be prudent.

**Cytoplasmic maturation**

Events comprising oocyte cytoplasmic maturation are less well defined than nuclear maturation, but equally essential. Cytoplasmic maturation entails proper relocation of organelles, synthesis of proteins, and post-translational modifications of mRNAs accumulated during oogenesis required for successful completion of meiosis, subsequent fertilization and preimplantation embryo development (Smith, 2001). Intra-oocyte processes regulating these events have been further classified by some as ‘molecular maturation’ (Sirard et al., 2006). Importantly, markers of successful cytoplasmic maturation are also not readily visualized, thus making judgment of oocyte quality difficult.

**Meiotic versus developmental competence**

It is important to note that nuclear and cytoplasmic maturation are normally a coordinated event, with GVBD releasing nuclear contents into oocyte cytoplasm. This undoubtedly has some affect on cytoplasmic maturation. However, although nuclear maturation can influence cytoplasmic components, at least some aspects of cytoplasmic maturation are independent of the nuclear portion. As an example, development of embryos from 2-cell to blastocyst stage is significantly higher when derived from MII oocytes from 26-day-old mice, compared with 18-day-old mice (Eppig et al., 1994). Thus, although oocytes may appear meiotically mature in regard to nuclear maturation and display extrusion of the first polar body, depending on their degree of cytoplasmic maturation, these oocytes may actually be lacking essential maternal factors required for subsequent fertilization, PN formation and embryo development. Therefore, it is beneficial to think of oocyte maturation as the process of an oocyte developing both meiotic and developmental competence (Eppig et al., 1994; Eppig, 1996). In a stepwise fashion associated with growth, oocytes acquire the ability to develop to MII, while concomitantly developing the ability to become fertilized and form a healthy embryo. Together, these concepts make it readily apparent why simple visualization of oocytes gives us an incomplete picture as to their maturation status, and any conclusions drawn to that effect are simply assumptions. This further reinforces the necessity to delve deeper into regulatory mechanisms and pathways involved in oocyte maturation before pursuing explanations for fertilization failure.
Regulation of oocyte maturation

Follicular environment

As we are all aware, oocyte maturation is an extremely complex process whose regulation begins during its growth within the follicular environment. Ovarian follicles provide a microenvironment to foster oocyte growth and development, and are responsible for production of hormones and secretion of growth factors intimately involved in regulation of oocyte meiosis. Within this follicular environment, oocytes are also subject to intricate interactions with surrounding granulosa/cumulus cells. Cumulus cells are responsible for providing nutritional requirements to oocytes, as well as relaying important chemical and molecular signals involved in regulating oocyte maturation via gap junction connections (Picton et al., 2007). Therefore, oocyte maturation and quality are influenced not only by factors acting directly on the female gamete, but also by regulators of the follicular environment. Clearly, this is indicated by the effects of exogenous gonadotrophins used in ovarian stimulation to alter oocyte metabolism (Fagbohun and Downs, 1992; Zuelke and Brackett, 1992; Downs et al., 1996; Roberts et al., 2004), ATP content, transcription (Combelles and Albertini, 2003), degeneration (Yun et al., 1987, 1989), asynchronous nuclear/cytoplasmic maturation (Yun et al., 1987, 1989), microfilament distribution (Lee et al., 2005, 2006), oocyte-derived embryo polyplody (Maudlin and Fraser, 1977; Sato and Marrs, 1986; Ma et al., 1997) and developmental competence (Pellicer et al., 1989; Blondin et al., 1996; Ertzeid and Storeng, 2001; Van der Auwera and D’Hooghe, 2001; Cheon et al., 2004; Racowsky et al., 2005; Andersen et al., 2006). Furthermore, it is also important to remember that supranumary oocytes retrieved for ART are not all destined to ovulate without the assistance of ovarian stimulation. Approximately 300,000 oocytes are present at the time of menarche and can range from 10,000 to total depletion at menopause. If one assumes the average age of menarche to be 13, and the average age of menopause to be 50, oocyte decline expressed on a monthly basis approaches 650. Of course, loss is not linear but there is still extensive loss throughout a woman’s reproductive lifespan and some loss occurs after follicles gain gonadotrophin responsiveness. Thus, IVF cycles are likely dealing with inherently compromised oocytes, which may offer insight into failed fertilization.

Molecular regulation

Complexity of oocyte meiotic regulation is overwhelming when considering the plethora of involved signaling pathways. For example, it is well known that during growth oocytes begin extensive accumulation of mRNAs and proteins. As mentioned, these are essential steps for supporting oocyte maturation, fertilization and initial preimplantation embryo cleavage prior to activation of the embryonic genome. However, fully grown oocytes are transcriptionally inert, or at least largely repressed (De La Fuente and Eppig, 2001). Rather, fully grown oocytes rely on protein synthesis and degradation as a means to control their maturation, a process heavily reliant upon polyadenylation (Eichenlaub-Ritter and Peschke, 2002) or destruction/maintenance of stored transcripts (Su et al., 2007). Additionally, oocyte maturation is heavily regulated via reversible phosphorylation of proteins, which often results in activation or inactivation of proteins and signaling.

**Figure 1:** Representative images composed by the authors of immunocytochemically stained oocytes at various meiotic stages to demonstrate chromatin and microtubule dynamics during oocyte nuclear maturation. These images serve to reinforce the fact that simple visualization of polar body extrusion does not indicate fidelity of oocyte maturation, as unseen chromosomal or meiotic spindle defects may be present. Microtubules are stained with β-tubulin (green), while chromatin is stained with Hoescht (blue). GVBD and metaphase I images were taken at ×100. Anaphase, telophase and metaphase II images were taken at ×93.
Oocyte-derived IVF/ICSI failure

As mentioned, despite retrieval of ‘normal’ appearing MII oocytes and use of quality sperm of known fertility, fertilization failure remains an issue in ART. Etiologies of fertilization failure in clinical IVF and ICSI have been outlined previously (Selva et al., 1991; Plachot and Crozet, 1992; Dozortsev et al., 1994; Asch et al., 1995; Flaherty et al., 1995; Wall et al., 1996; Gook et al., 1998; Rawe et al., 2000), which result in absence of PN, presence of only one PN (male or female) or presence of multiple PN (Table I). However, since these early studies, significant progress and discovery has been made in understanding oocyte regulatory mechanisms governing fertilization events that may provide insight into potential causes of fertilization failures, as well as potential therapies.

To begin to understand possible causes of these fertilization failures, it is essential to consider sperm–oocyte interactions during IVF. As we are all aware, in the clinical embryology laboratory, sperm must traverse the surrounding cumulus cells, bind to a mature oocyte, penetrate the zona pellucida and fuse with the oolemma. Subsequently, the oocyte activates, allowing sperm processing within oocyte cytoplasm, culminating in PN formation. From this brief description, and for purpose of the following discussions, we have chosen to divide oocyte fertilization into six major events:

(i) Cumulus cell penetration
(ii) Sperm/oocyte binding and penetration
(iii) Sperm/oocyte fusion
(iv) Oocyte activation
(v) Sperm processing
(vi) PN formation

The first three of these mechanistic events, or pre-sperm penetration, can be bypassed in the embryology laboratory via common techniques such as ICSI. However, fertilization failure still occurs despite microinjection of sperm, indicating that sperm penetration is not the overall problem (Fig. 3) (Flaherty et al., 1995; Gook et al., 1998). Regulation of post-sperm penetration events is at least as essential in successful fertilization as pre-sperm penetration events (Table II). Importantly, improper or inadequate oocyte maturation can impede both pre and post-sperm penetration events and result in fertilization failure (Fig. 4, Table I). In the following paragraphs, we highlight oocyte controlled molecular and cellular events essential for completion of these six mechanistic fertilization steps.

Cumulus cell penetration

The first obstacle encountered by sperm in clinical IVF prior to reaching the oocyte is navigation through surrounding cumulus cells and their extracellular matrix (Fig. 5.1). The extracellular matrix of cumulus cells consists primarily of the glycosaminoglycan, hyaluronan, which forms a mesh-like network by cross-linking various proteins and proteoglycans (see review Russell and Salustri, 2006). Formation of this matrix results in the phenomenon commonly referred to as cumulus expansion or mucification.

Well-established data from animal studies indicate oocyte-secreted paracrine factors can regulate cumulus cell differentiation (Vanderhyden et al., 1990; Li et al., 2000) and expansion (Buccione et al., 1990; Salustri et al., 1990a, b; Dragovic et al., 2005, 2006; Gui and Joyce, 2005; Gilchrist et al., 2006). This expansion appears to be due to increased hyaluronan synthesis (Salustri et al., 1990a, b), possibly through secretion of oocyte growth differentiation factor-9 (GDF-9) (Vanderhyden et al., 2003; Su et al., 2004; Dragovic et al., 2005; Gui and Joyce, 2005), bone morphogenic protein-15 (BMP-15 (Su et al., 2004; Gueripel et al., 2006) or another activator of the SMAD 2/3 signaling pathway (Dragovic et al., 2006). Additionally, oocyte secreted BMP-15 prevents cumulus cell apoptosis (Hussein et al., 2005). Increased cumulus cell apoptosis is correlated with lower fertilization rates during clinical IVF (Nakahara et al., 1997; Host et al., 2000, 2002; Lee et al., 2001). Furthermore, oocytes can direct cumulus cell steroidogenic activity (Vanderhyden and Macdonald, 1998; Li et al., 2000) and metabolism (Zuelke and Brackett, 1992; Sugiuira et al., 2005, 2007; Su et al., 2008). Oocyte-directed effects on cumulus cell growth and function appear to be due, in part, via regulation of cumulus cell transcript levels (Vanderhyden et al., 2003; Su et al., 2004; Dragovic et al., 2005; Gui and Joyce, 2005; Diaz et al., 2007a, b). Thus, cumulus cells may be reflective of health of their enclosed oocyte. Indeed, emerging research indicates the cumulus cell transcriptome may be a valid predictor of oocyte fertilization potential and developmental competence (McKenzie et al., 2004; Cillo et al., 2007; Hamel et al., 2008).

Thus, even though cumulus oocyte complexes appear ‘normal’, aberrant maturation may result in abnormal secretion of oocyte factors, such as GDF-9 or BMP-15, essential for proper cumulus cell growth and function. This altered cumulus cell environment has the potential to not only impair sperm transit, but also to compromise accompanying sperm processes associated with this passage essential for fertilization, such as capacitation or potentiation of the sperm acrosome reaction (Tesarik, 1985; Sabeur et al., 1998). Though procedures such as cumulus cell removal and ICSI can circumvent this particular issue, it does not address the underlying defects responsible.

Sperm/oocyte binding and penetration

After traversing surrounding cumulus cells, spermatozoa encounter and bind, then penetrate the zona pellucida. The zona pellucida is a conglomerate of glycoproteins synthesized and secreted by
growing oocytes, which increases in thickness as oocytes increase in diameter (Bleil and Wassarman, 1980; Greve et al., 1982). These proteins are synthesized as large glycosylated polypeptide chains, which require further processing and proteolytic cleavage prior to secretion by Golgi bodies and subsequent polymerization (Bleil and Wassarman, 1980; Greve et al., 1982; Litscher et al., 1999; Williams and Wassarman, 2001; Kiefer and Saling, 2002) (Fig. 5.2). Studies utilizing mouse oocytes indicate major constituents of zona

Figure 2: Mechanistic events of oocyte nuclear maturation and associated protein kinases/phosphatases regulating molecular and cellular signaling pathways responsible. AURKB, aurora B kinase; AURKA, aurora A kinase; CDC25, cell division cycle 25 phosphatase; CDK1, cyclin-dependent kinase1; PP1, protein phosphatase-1; PP2A, protein phosphatase 2A; GSK3, glycogen synthase kinase 3; PLK1, polo-like kinase 1; MAPK, mitogen-activated protein kinase; PKA, protein kinase A; PKC, protein kinase C.
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Table 1. Summary of failed fertilization etiologies and their prevalence as listed in the literature following human IVF and ICSI.

<table>
<thead>
<tr>
<th>Etiology</th>
<th>Prevalence, %</th>
<th>Manifestation</th>
<th>References</th>
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</thead>
<tbody>
<tr>
<td>Pre-sperm penetration</td>
<td></td>
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<td></td>
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<tr>
<td>No sperm incorporation</td>
<td>15–56</td>
<td>No PN, single female PN</td>
<td>Asch et al. (1995), Rawe et al. (2000)</td>
</tr>
<tr>
<td>Maternal chromosomal defects</td>
<td>10–30</td>
<td>No PN, Multiple female PN</td>
<td>Asch et al. (1995), Selva et al. (1991)</td>
</tr>
<tr>
<td>Post-sperm penetration</td>
<td></td>
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<tr>
<td>Failed oocyte activation</td>
<td>15–66</td>
<td>No PN, 3 PN due to absence of 2nd PB extrusion</td>
<td>Rawe et al. (2000), Flaherty et al. (1995)</td>
</tr>
<tr>
<td>Failed sperm head decondensation</td>
<td>4–45</td>
<td>No PN, single female PN</td>
<td>Flaherty et al. (1995), Gook et al. (1998), Dozortsev et al. (1994)</td>
</tr>
<tr>
<td>condensation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spindle defects/sperm aster</td>
<td>6–18</td>
<td>No PN, failed apposition of PN</td>
<td>Asch et al. (1995), Rawe et al. (2000)</td>
</tr>
<tr>
<td>defects</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polyspermic penetration</td>
<td>3–9</td>
<td>Multiple PN</td>
<td>Asch et al. (1995), Plachot and Crozet (1992)</td>
</tr>
<tr>
<td>Sperm ejection</td>
<td>6–23</td>
<td>No PN, single female PN</td>
<td>Rawe et al. (2000), Flaherty et al. (1995), Gook et al. (1998)</td>
</tr>
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</table>

Sperm binding of oocyte ZP3 elicits the sperm acrosome reaction, facilitating binding of ZP2, subsequently allowing penetration of the zona pellucida. Based on inability of human sperm to bind mouse oocytes expressing human ZP3 (Rankin et al., 1998), more recent models suggest that, rather than dependence on just ZP3, binding of human sperm to oocytes is dependent upon the supramolecular structure of all four glycoproteins, with an important role for ZP4. However, although rat oocytes contain four ZP glycoproteins, similar to human, these oocytes still cannot bind human sperm (Hoodbhoy et al., 2005). Thus, it is evident that simple presence of ZP glycoproteins is not sufficient to elicit sperm binding and convey species specificity.

Species-specific sperm recognition and binding of zona pellucida appears to be facilitated by carbohydrate moieties located on the exterior of the glycoprotein shell. Specific O-linked oligosaccharides associated with ZP3 are instrumental in mouse sperm/zona recognition and binding (Florman et al., 1984; Florman and Wassarman, 1985). Expression of N-linked glycans has also been suggested to play a role in binding (Nagdas et al., 1994; Yonezawa et al., 1995; Nakano et al., 1996; Amari et al., 2001). Indeed, oxidation of human zona pellucida glycans reduces sperm binding, however, it does not prevent it completely (Ozgur et al., 1998). Coupled with additional data indicating knock-out mice lacking key oocyte O- and N-linked glycans are fertile (Ellies et al., 1998; Shi et al., 2004), these data suggest presence of a redundancy system involved in sperm/zona recognition and binding; involving protein–protein, carbohydrate–protein or a yet unidentified system (see review Clark and Dell, 2006).

Notably, scanning electron microscope studies indicate structure of mouse zona pellucidae change during oocyte maturation (Nogues et al., 1988; Calafell et al., 1992), displaying variations in localization, density and distributions of glycoconjugates (Kaufman et al., 1989), Ultrastructural changes in zona pellucida of human oocytes also occur depending on maturational status (Familiari et al., 1988, 1989, 1992; Tesarik et al., 1988). It is likely these changes to surface ultrastructure of zona pellucidae are important in regulating sperm/oocyte binding, perhaps via orientation of sperm-binding sites (Familiari et al., 1988). Indeed, anomalies in ZP3 structural backbone of human oocytes, as evidenced by reduced antibody staining, appears to be at least

Figure 3: Differential interference contrast image of failed fertilization in a human oocyte following IVF, despite presence of a spermatozoan in cytoplasm. Although oocytes may obtain complete nuclear maturation, they may be cytologically immature, lacking essential factors for successful fertilization. These same deficiencies responsible for failed fertilization post-sperm penetration are also present following ICSI. Image was taken at × 100 ~ 18 h following insemination.
### Table II. List of mechanistic fertilization events and possible oocyte deficiencies resulting in failed fertilization.

<table>
<thead>
<tr>
<th>Fertilization event</th>
<th>Possible oocyte-derived causes of fertilization failure</th>
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<tbody>
<tr>
<td>Cumulus cell penetration</td>
<td>Aberrant GDF-9 or BMP-15 secretion, resulting in abnormal cumulus differentiation or increased apoptosis, affecting sperm penetration or acrosome reaction.</td>
</tr>
<tr>
<td>Zona binding</td>
<td>Abnormal production or secretion of ZP1, ZP2, ZP3, or attached carbohydrate moieties; resulting in altered zona pellucida structure and inability to bind sperm or elicit acrosome reaction.</td>
</tr>
<tr>
<td>Fusion</td>
<td>Inadequate oolema receptor expression (CD9 or GPI proteins), inappropriate microvilli distribution, or lack of essential signaling proteins, resulting in lack of membrane conformational changes and gamete membrane fusion events.</td>
</tr>
<tr>
<td>Activation</td>
<td>Inadequate or abnormal Ca(^{2+}) oscillations caused by malfunctioning endoplasmic reticuli, mitochondria, or IP3 signaling pathways, resulting in aberrant kinase/phosphatase signaling cascades, leading to failed cortical granule exocytosis or failure to correctly complete the second meiotic division.</td>
</tr>
<tr>
<td>Sperm processing</td>
<td>Failed sperm head decondensation due to failed fusion or activation events or abnormal reversible phosphorylation. Failure to remodel chromatin due to insufficient stores of oocyte-derived proteins (glutathione, histones)</td>
</tr>
<tr>
<td>Pronuclear formation</td>
<td>Insufficient oocyte stores of membrane vesicles, ATP/GTP or lamin B can result in failure to form pronuclei. Defective chromatin remodeling can result in multiple pronuclei. Additionally, aberrant reversible phosphorylation and spindle formation/function can result in failed pronuclear migration.</td>
</tr>
</tbody>
</table>

GDF-9, growth differentiation factor-9; BMP-15, bone morphogenic protein-15; GPI, glycosylphosphatidylinositol anchored proteins; ZP1, 2, 3, ZP glycoproteins of zona pellucida; CD9, oolemma tetraspanin protein; IP3, inositol triphosphate.

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Figure 4: Diagram of six steps required for successful fertilization.

Step 1 entails sperm penetration of expanded cumulus cells. Step 2 requires sperm recognition of the zona pellucida, dependent upon the four ZP proteins (ZP1, 2, 3, 4), as well as linked oligosaccharides. (a) Sperm bind ZP3, (b) undergo the acrosome reaction and bind ZP2, (c) becoming competent to penetrate the zona pellucida. Step 3 involves sperm/oocyte fusion events where (a) the sperm bind the oolemma through interactions with microvilli and associated membrane proteins and (b) subsequently form a fusion pore. Step 4 encompasses the process of oocyte activation, where a signaling cascade leads to the cortical granule reaction and block to polyspermy through modification of ZP2 and ZP3, as well as completion of the second meiosis and extrusion of the 2 polar body. Step 5 includes processes required for processing of the sperm to release its nuclear contents. Step 6 completes the fertilization process through formation of the PN and their migration as they prepare for syngamy.

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Oocyte-derived fertilization failure

one cause of reduced sperm binding and oocyte-derived fertilization failure during IVF (Oehninger et al., 1996).

Therefore, aberrant oocyte growth and maturation affecting signaling pathways involved in zona pellucida glycoprotein production, processing or secretion, could result in fertilization failure due to abnormal sperm recognition/binding. Interestingly, exogenous gonadotrophins have been reported to alter the structure of zona pellucida (Katzberg and Hendrickx, 1966; Marullo, 1967) and may influence capacity to bind sperm in human oocytes. Alternatively, aberrant zona pellucida composition or ultrastructure could result in fertilization failure due to inability to elicit sperm acrosome reaction (Beebe et al., 1992; Henkel et al., 1998). Regardless, as with cumulus cell penetration, techniques such as ICSI offer a means to bypass this potential fertility issue.

### Sperm/oocyte fusion

Following zona pellucida binding and traversing of the perivitelline space, spermatozoon approach the oolemma. Electron microscopy reveals that this outermost membrane of human oocytes are variably covered in microvilli, with cells showing either an overabundance of evenly distributed microvilli, sparse patches or a complete lack of microvilli (Schwartz et al., 2003). These microvilli are thought to play a role in sperm/oocyte fusion, as microvilli surround sperm during fusion (Yanagimachi, 1978; Shalgi and Phillips, 1980; Longo and Chen, 1984). Subsequently, through a series of complex molecular interactions, the sperm membrane must adhere to the oolemma, which results in fusion of gamete membranes. Adhesion is proposed to be mediated by receptor-ligand pairs located on the sperm and oocyte, with one pair being primarily responsible for adhesion and the other pair binding gamete membranes into closer apposition. Subsequently, a conformational change in an unspecified fusion peptide is initiated, which inserts into the opposing gamete bilayer, culminating in opening and expansion of a fusion pore (Evans, 2002) (Fig. 5.3).

Although identification of these proposed receptor-ligand pairs and fusion proteins on sperm and oocytes are not resolved, it appears as though the oolemma tetraspanin protein, CD9, is critical for sperm/oocyte fusion. CD9 is distributed over the surface of oolemma, appearing around the time oocytes become competent to bind sperm (Komorowski et al., 2006) and enriched in areas of microvilli (Runge et al., 2007). Additionally, both use of neutralizing CD9-antibody (Chen et al., 1999) and CD9-deficient mouse models (Kaji et al., 2000; Miller et al., 2000; Miyado et al., 2000) result in reduced or absent sperm/oocyte fusion. Interestingly, tetraspanin proteins are thought to form multi-molecular
complexes with various plasma membrane-associated proteins, including integrins (see review Boucheix and Rubinstein, 2001). Previous models suggested integrins on oolemma were instrumental in sperm binding and fusion, through interactions with sperm-associated A Disintegrin and Metalloprotease (ADAMS), such as fertilin (see reviews Evans, 2001; Kaji and...
Kudo, 2004). However, more recent findings utilizing integrin knock-out mice indicate these proteins are not required for sperm/oocyte binding or fusion (Miller et al., 2000; He et al., 2003). Alternative oolemma-associated proteins that appear to function in the fusion process include glycosylphosphatidylinositol (GPI)-anchored proteins. Oocyte-specific knockout of GPI protein biosynthesis results in infertility and dramatic reduction in sperm binding (Alfieri et al., 2003).

Thus, failed sperm/oocyte fusion is another possible event responsible for fertilization failure. This may be due to improper microvilli distribution patterns of microvilli, possibly due to aberrant CD9 expression (Runge et al., 2007). Indeed, oocytes displaying reduced sperm fusion demonstrated alterations in structure and number of microvilli (Runge et al., 2007). Microvilli distribution appears to be a dynamic process reflective of maturation status and possibly quality of the female gamete (Suzuki et al., 1981; Sezen and Cincik, 2003; Cecconi et al., 2006). Distribution of microvilli appears to be regulated, in part, via estrogen levels during development (Zachos et al., 2004), and thus may be impacted by controlled ovarian stimulation protocols. Additionally, improper oolemma protein content or orientation may prevent gamete membrane fusion events.

Oocyte activation
Following sperm/oocyte fusion, oocytes undergo a series of morphological and biochemical alterations in a process known as activation. Oocyte activation is an enigmatic process, but is usually categorized as entailing two major events, including (i) modifications of zona pellucida to prevent polyspermy (cortical granule reaction) and (ii) release from MII arrest and completion of the second meiotic division. Concomitant with these mechanistic events, oocytes experience changes in protein synthesis essential for subsequent fertilization events.

A rapid increase in intracellular calcium (Ca\(^{2+}\)) levels, followed by rapid and repetitive oscillations, is one of the first responses following sperm/oocyte fusion indicative of oocyte activation. These oscillations begin a few minutes after gamete fusion and cease around the time of PN formation, perhaps due to nuclear sequestration of a Ca\(^{2+}\)-releasing agent (Marangos et al., 2003; Larman et al., 2004). Frequency, amplitude and duration of the Ca\(^{2+}\) rise and oscillations appear to have dramatic effects on subsequent fertilization events and embryo development (Ozil, 1998; Gordo et al., 2000; Ducibella et al., 2002; Rogers et al., 2006), perhaps as a result of altered gene expression (Ozil et al., 2006). This may be due to dependence upon a minimal threshold, requiring summation of Ca\(^{2+}\) signals (Toth et al., 2006). Ability to produce Ca\(^{2+}\) oscillations is acquired as oocytes reach full size, but complete oscillation capability is only acquired following successful oocyte maturation (Carroll et al., 1994). Calcium originates from endoplasmic reticuli within oocytes and, in human, this Ca\(^{2+}\) spike and following oscillations appear to emanate outward in a wave-like pattern from the site of sperm entry, with oscillations occurring anywhere from 10 to 35 min (Taylor et al., 1993; Tesarik and Sousa, 1994). Although three main theories exist for explaining how sperm induce Ca\(^{2+}\) oscillations within oocytes; including the Ca\(^{2+}\) conduit hypothesis and the receptor-mediated model (see review Malcuit et al., 2006), accumulating evidence in human supports the theory that sperm introduce a cytosolic component directly into oocyte cytoplasm to trigger oscillations (Homa and Swann, 1994). This sperm component appears to be a sperm-specific phospholipase C (PLC zeta), which activates the inositol triphosphate (IP3) receptors and signaling pathways to elicit release of intra-oocyte Ca\(^{2+}\) stores and facilitate oscillations (Cox et al., 2002; Swann et al., 2004) (Fig. 5.4). Importantly, redistribution of oocyte endoplasmic reticuli and IP3 receptors corresponds to maturation status of oocytes as they develop the ability to produce sperm-induced Ca\(^{2+}\) oscillations (Mehlmann et al., 1995, 1996). Additionally, oocyte mitochondria display polarization associated with their maturation status and this distribution is also important for proper cellular functioning (Van Blerkom and Runner, 1984; Van Blerkom et al., 2002). Emerging data suggest oocyte mitochondria play an instrumental role in interactions with endoplasmic reticulum and IP3 Ca\(^{2+}\) signaling (Dumollard et al., 2006). Sperm-induced Ca\(^{2+}\) oscillations stimulate mitochondrial respiration, and the resulting mitochondrial ATP production is required to maintain sperm-triggered Ca\(^{2+}\) waves (Dumollard et al., 2004). It is theorized that, similar to somatic cells, oocyte mitochondria may play a dynamic role in regulating activation-induced Ca\(^{2+}\) signaling by sequestering and buffering cytoplasmic Ca\(^{2+}\) (Dumollard et al., 2006).

Cortical granule reaction
As mentioned, Ca\(^{2+}\) oscillations induce several activation events. One such event involves specialized organelles, known as cortical granules. In response to Ca\(^{2+}\) rise, cortical granules migrate toward the oolemma to release their enzyme contents into the perivitelline space in a process known as the cortical reaction (Sathananthan et al., 1985; Sathananthan, 1994). Cortical granule release results in alteration of zona pellucida, cleaving ZP2 to ZP2f, and modifying ZP3, thus preventing further sperm binding and polyspermic penetration (Ducibella et al., 1990; Schroeder et al., 1990). Indeed, electron microscopy shows that while unfertilized oocytes contain a highly textured zona pellucida, following fertilization, the zona pellucida becomes smoother and less porous (Jackson and Dumont, 1979).

Evidence from several studies indicate Ca\(^{2+}\)-induced cortical granule release results from activation of G-coupled proteins, which activate the inositol phosphate (PIP2) cascade, resulting in production of IP3 and diacylglycerol (DAG) (see reviews Ducibella, 1996; Sun, 2003). DAG can activate downstream signaling events important for cortical granule release in human oocytes, such as activation of protein kinase C (PKC) (Wu et al., 2006) (Fig. 5.4).

Importantly, ability of oocytes to undergo cortical granule exocytosis is acquired during the maturation process, as demonstrated by the inability of pre-ovulatory mouse oocytes to complete exocytosis in response to Ca\(^{2+}\) oscillations (Abbott et al., 1999). This inability to complete the cortical reaction is not due to inadequate number of cortical granules, or due to inappropriate intracellular localization, but rather appears to be the result of an inability to respond to Ca\(^{2+}\) oscillations and translocate to the cortex (Abbott et al., 2001).

Completion of meiosis II
Completion of the second meiotic division is another important event encompassed by the process of oocyte activation.
Completion of meiosis II entails resumption of meiosis from MII, separation of sister chromatids, and extrusion of the second polar body. Assessment of clinical IVF failures indicate that failure to complete these processes correctly can result in presence of 3PN due to retention of extra maternal genetic material (2 maternal PN) (Flaherty et al., 1995), formation of multiple small female PN due to inappropriate segregation of chromosomes (karyomeres) (Asch et al., 1995) or complete absence of the female pronucleus (Asch et al., 1995). Completion of the second meiosis in oocytes also appears to be dependent, in part, upon Ca\textsuperscript{2+} oscillations. These oscillations are implicated in activation and/or inactivation of a variety of signaling pathways involving various protein kinases and phosphatases. These enzymes play instrumental roles in regulation of machinery required for oocyte meiosis. As an example, Ca\textsuperscript{2+} oscillations appear to be instrumental in destruction of cytotstatic factor (CSF), possibly through activation of Ca\textsuperscript{2+}-dependent calmodulin-dependent kinase II (CaMKII) (Lorca et al., 1993). CSF is the name given to the cytoplasmic component of oocytes responsible for maintaining MII arrest (Masui and Markert, 1971). It is now known that CSF consists of Mos protein, which regulates mitogen-activated protein kinase (MAPK) activity, and stabilizes maturation promotion factor (MPF) (see review Tunquist and Maller, 2003). Decreased MAPK (Gordo et al., 2004) and MPF activities (Hashimoto and Kishimoto, 1986; Naito and Toyoda, 1991; Kikuchi et al., 1995) are requirements for MII meiotic resumption. MAPK is also just one of several key enzymes regulating actin microfilaments and meiotic spindle components important for sister chromatid segregation and polar body extrusion (see reviews Fan and Sun, 2004; Swain and Smith, 2007a, b) (Fig. 5.4). Oocyte MPF is intimately involved with separation of sister chromatids via interactions with the cohesin complex, which holds chromatids together until anaphase (Madgwick et al., 2004); a process also dependent upon balanced activity of additional kinases and phosphatases; including polo-like kinase 1 and aurora kinases (Swain and Smith, 2007a, b).

In summary, importance for oocytes to correctly produce and regulate Ca\textsuperscript{2+} oscillations for successful oocyte activation is readily apparent, as ultimately, regulation of a few Ca\textsuperscript{2+}-dependent kinases/phosphatase signaling pathways has a tremendous impact due to a cascade effect and activation/inactivation of a multitude of other enzymes. These enzymatic pathways regulate most processes associated with completion of oocyte maturation and fertilization events. Inadequate or inappropriate oocyte cytoplasmic maturation can result in abnormal fertilization due to insufficient protein synthesis or aberrant signaling pathways, resulting in cortical reaction failure and polyspermy penetration. Alternatively, oocytes can also undergo premature cortical granule exocytosis and zona hardening, resulting in inability of sperm to penetrate the oocyte. Interestingly, failure of sperm to penetrate the oocyte is the major cause of failed fertilization following IVF. Thus, besides previously mentioned potential considerations (see sperm penetration), premature oocyte activation prior to sperm penetration may actually be the underlying causative factor. Though microinjection of sperm can avoid this limitation, it should be noted that failed oocyte activation is the major cause of fertilization failure in human oocytes following ISCI (Flaherty et al., 1995). Thus, factors compromising Ca\textsuperscript{2+} oscillations and associated signaling cascades involved in oocyte activation can subsequently alter mechanistic processes such as sister chromatid separation, polar body extrusion and other critical meiotic events. In some instances, oocyte-derived causes of activation failure may be circumvented through modification of injection technique (Tesarik et al., 2002; Ebner et al., 2004). Other approaches employ artificial activation. Through the use of various Ca\textsuperscript{2+} ionophores or protein synthesis inhibitors, clinics have been able to rescue oocyte activation failure to obtain pregnancies (Eldar-Geva et al., 2003; Chi et al., 2004; Heindryckx et al., 2005). However, again, caution should be exercised with these rescue techniques, as developmental competence of resulting embryos may be compromised and long-term ramifications on offspring are unknown.

Sperm processing

Coincident with oocyte activation events, spermatozoa undergo biochemical remodeling dependent upon endogenous resources within cytoplasm of oocytes. This processing entails incorporation, modification and even decomposition of sperm components as paternal chromatin is remodeled. Initial sperm processing entails decondensation of sperm heads, which releases sperm nuclear contents into cytoplasm of oocytes. Decondensation occurs in three main stages prior to PN formation; including (i) removal of poreless sperm nuclear membranes, (ii) disassembly of the underlying structural lamina and (iii) decondensation of chromatin (see reviews Poccia and Collas, 1996, 1997). Additionally, oocytes process the sperm-inherited centrosome and provide essential proteins such as gamma tubulin to aid in formation of the sperm aster (Schatten, 1994), which is important for subsequent organization of the spindle apparatus necessary for PN migration. Time lapse imaging of human oocytes demonstrates these events occur immediately prior to extrusion of the second polar body (Payne et al., 1997).

Very little is known regarding initial events of oocyte controlled sperm processing. It is thought removal of sperm nuclear membranes may be facilitated during sperm/oocyte fusion, although exact mechanisms are unknown (Poccia and Collas, 1997). Disassembly of sperm lamina is dependent upon reversible phosphorylation (see section PN formation), possibly by PKC (Collas et al., 1997). However, more is known regarding remodeling of sperm chromatin. Chromatin of mammalian spermatozoa is organized by protamines and is extremely compact and transcriptionally inactive. Oocyte-derived factors facilitate decondensation of paternal chromatin through reduction of protamine disulfide bonds. Via reversible phosphorylation, sperm chromatin subsequently recondense as protamines are replaced with maternally derived histones from within oocyte cytoplasm (McLay and Clarke, 1997; McLay et al., 2002). Furthermore, oocyte cytoplasmic-derived factors also control epigenetic modifications to paternal DNA, such as methylation. (Oswald et al., 2000) (Fig. 5.5).

Ability of oocytes to process sperm components and remodel paternal chromatin is dependent upon their maturation status. The concentration of oocyte glutathione increases during maturation and is required for sperm decondensation and formation of the male pronucleus (Perreault et al., 1988; Sutovsky and Schatten, 1997). Furthermore, premature sperm chromatin condensation (PCC) is a prevalent etiology of fertilization failure in human ART and PCC is dependent upon the maturity of the oocyte (Calafell et al., 1991), possibly as the result of inability to complete
octoyte activation (Nasr-Esfahani et al., 2007). Capability of oocytes to replace sperm protamines with histones is gained during maturation. Indeed, studies indicate Ca\(^{2+}\) oscillations promote histone assembly onto paternal chromatin (McLay et al., 2002), and these oscillation are a hallmark of oocyte maturity and activation.

Thus, despite bypassing initial fertilization events by directly injecting sperm into oocyte cytoplasm via ICSI, incomplete accumulation of oocyte cytoplasmic proteins; such as, histones or glutathione, can compromise regulatory pathways and still result in fertilization failure due to improper processing of sperm components.

**PN formation/apposition**

Formation of male and female PN marks the completion of mammalian fertilization. PN formation involves re-establishment of the NE around the respective genetic material from both the sperm and oocyte. This process begins with fusion of membrane vesicle containing lamina receptors, followed by incorporation of nuclear pore complexes into the forming NE, followed by transport of lamins through pores to form underlying nuclear lamina scaffold (Macaulay and Forbes, 1996). Time-lapse cinematography indicates male PN form centrally within the human oocyte, slightly earlier or simultaneously with female PN, which forms adjacent to the second polar body (Payne et al., 1997). The female pronucleus is then drawn centrally toward the male pronucleus via sperm aster microtubules organized by the centrosome introduced by the sperm (Schatten, 1994). The male pronucleus subsequently increases in size to become slightly larger than the female. This growth or swelling of the male pronucleus appears to be largely due to aggregation and fusion of oocyte-derived vesicle membranes and addition of soluble lamin B; a process dependent upon GTP and ATP (Collas and Poccia, 1998), perhaps generated from increased glucose metabolism (Comizzoli et al., 2003; Urner and Sakkas, 2005) (Fig. 5.6).

NEs consist of an inner and outer membrane overlying a network of filament-type proteins known as nuclear lamins, whose formation is intimately regulated via interactions with chromatin (see review Sturman et al., 1998). Mammalian oocytes contain all three lamins, A, B and C. (Schatten et al., 1985; Maul et al., 1987; Houliston et al., 1988) and NE integrity is controlled through phosphorylation and dephosphorylation of these nuclear lamins. Hypo-phosphorylation of nuclear lamins maintains NE integrity, whereas hyper-phosphorylation of lamins results in NE disassembly (Sturman et al., 1998). MAPK activity appears essential for mouse PN assembly following fertilization (Moos et al., 1995), while oocyte-derived PKC is also suggested to play a critical role in regulation of lamin B phosphorylation and sperm NE integrity following fertilization in sea urchin (Collas et al., 1997). A complex containing CDK1 kinase and cyclin B, similar to MPF, is implicated in disassembly of clam oocyte NE (Dessev et al., 1991), while protein phosphatase-1 (PP1) plays a role in regulating mouse oocyte NE integrity during meiosis I (Swain et al., 2003). Therefore, regulation of these enzymes may also play a role in NE reassembly during PN formation (Fig. 5.6). Thus, it is readily apparent NE integrity and PN formation is determined through a balanced activity of several oocyte-derived protein kinase and phosphatase interactions.

Aside from formation of multiple PN due to polyspermic penetration, assessment of abnormal fertilization indicates PN can fail to form, fail to migrate or fail to grow (Payne et al., 1997). Failure to form PN can result from disruptions in meiotic chromatin segregation and subsequent failure to correctly regulate necessary signaling pathways for lamin assembly. Failure to properly assemble NE constituents, such as nuclear pore complexes, has been associated with PN arrest in human zygotes (Rawe et al., 2003). Alternatively, inadequate stores of oocyte-derived proteins or substrates, such as soluble lamin B, or insufficient energy stores of membrane vesicles can compromise PN formation. Accumulation of these factors is achieved throughout oocyte maturation. Indeed, immature MI human oocytes were found to be lacking unknown factors required for PN formation, compared with their mature MI counterparts (Tesarik and Kopecky, 1989). Furthermore, even with successful PN formation, defective sperm aster formation, possibly as the result of inappropriate processing by the oocyte, can negatively impact PN migration and prevent subsequent syngamy.

**Oocyte biology and indications for ICSI**

An understanding of the fundamental contributions of the oocyte to both fertilization and subsequent embryo developmental capacity is essential for identifying the proper indications and applications of ICSI. It is inarguable that ICSI is a cornerstone of ART, facilitating the ever increasing rates of success. Indeed, severe male factor infertility is the obvious candidate for ICSI. As well, history of previous failed IVF or absence of prior pregnancy, due either to male or female rooted causes, would elicit consideration for direct sperm injection to at least a portion of available oocytes. In these cases, ICSI may even provide limited insight into oocyte quality and maturation status, indicating possible causes of fertilization failure. In addition, application of ICSI for use in cases involving thawed oocytes, or PCR-based single gene detection PGD is probably justified. However, ICSI should not be applied universally, as the technique does not address all underlying oocyte-borne fertilization aberrations or uniformly circumvent fertilization failure, nor has it been shown that ICSI results in improved implantation or pregnancy compared with insemination. Furthermore, efficacy and safety of ICSI is often taken for granted, as invasiveness of the procedure is easily overlooked. Microinjection involves piercing of the oolemma, as well as avoidance of crucial unseen intra-oocyte organelles. Though the physical act of sperm microinjection is safe and does not result in any known abnormalities, it is a fact that intricacies and nuances involved in oocyte denuding, sperm immobilization and injection technique may actually hinder fertilization events if done incorrectly. Furthermore, these delicate practices not only influence fertilization success rates, but undoubtedly affect subsequent embryo development. Therefore, in conjunction with oocyte lysing, ICSI may actually impart added risk compared with standard IVF without justified reward. It may even be said that global application of ICSI serves to foster disconnection between clinical treatments, such as ovarian stimulation protocols, and resulting consequences imparted upon oocyte biology. Are oocytes that can only be fertilized via direct injection of sperm from normospermic patients really desirable? If not, then why treat all oocytes in this manner? Forgoing insemination in all cases...
prevents our ability to monitor certain aspects of oocyte maturation, as well as those variables that may be altering oocyte quality. Maintenance of strict selection criteria for ICSI will allow for further refinement of ART protocols to optimize oocytes quality and subsequently lead to improved outcomes.

Conclusion

Through supply of maternally-derived proteins and regulation of molecular and biochemical signaling pathways, the oocyte is largely responsible for regulating events involved in fertilization. Thus, although oocytes appear ‘normal’, non-visual abnormalities or incomplete oocyte maturation can result in failure at any one of these fertilization events; including sperm penetration of cumulus cells, binding zona pellucidae, fusion with oolemma, remodeling of paternal chromatin and PN formation. Indeed, many of these aberrations may be the result of inherent limitation within the oocyte, derived during growth and development, and thus destined to occur regardless of precautionary measures utilized within the laboratory. While our knowledge concerning the events of oocyte maturation has increased immensely, much about the process remains an enigma. It is increasingly evident that simple visualization is not an adequate assessment of oocyte quality and non-invasive methods to more efficiently assess oocyte maturation are required. This is especially important when considering the benefit and renewed interest in human oocyte IVM. Fortunately, progress has been made in this endeavor (see reviews Combelles and Racowsky, 2005; Krisher et al., 2007; Patrizio et al., 2007; Wang and Sun, 2007), and exciting data with potential predictive power of oocyte quality is emerging using oocyte respiration rates (Scott et al., 2006, 2007), as well as data from the metabolome (Nagy et al., 2007; Seli et al., 2007; Scott et al., 2008) and secretome (Bormann et al., 2006; Hussein et al., 2006; Katz-Jaffe et al., 2006). However, much remains to be done to elucidate markers of oocyte developmental competence before widespread implementation into clinical embryology laboratories.

Furthermore, the delicate nature of the oocyte and the instrumental role it plays regulating fertilization events reinforces the need to minimize stresses placed upon the female gamete in clinical ART. Although inherent qualities of the oocyte may largely dictate its fertilization fate, certainly, manipulations within the ART laboratory can compound these underlying issues, if not impart limitations of their own. Exposure to mechanical stressors during retrieval, cumulus cell removal or microinjection, as well exposure to sub-optimal temperature regulation, osmotic imbalances or large pH fluctuations, all have the potential to compromise oocyte developmental competence. While plenty of focus has been placed upon optimization of culture environment and reduction of stress to the preimplantation embryo, it is often easy to neglect the oocyte. This is an unwise philosophy, as a prerequisite to obtaining a healthy embryo is first obtaining a healthy oocyte. Thus, to continue to improve ART success rates, we must be vigilant in our quest to improve oocyte health through understanding of biological processes, as well as modification of stimulation protocols and further refinement of oocyte-specific culture conditions.

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Oocyte-derived fertilization failure


Swain and Pool


Swain and Pool


