

The effect of oocyte quality on development^{1,2}

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ABSTRACT: Oocyte quality affects early embryonic survival, the establishment and maintenance of pregnancy, fetal development, and even adult disease. Quality, or developmental competence, is acquired during folliculogenesis as the oocyte grows, and during the period of oocyte maturation. Assisted reproductive technologies involving ovarian hyperstimulation, or collection of immature oocytes followed by maturation in vitro, perturb this process and result in oocytes with reduced quality. In domestic livestock species, offspring have been produced using in vitro oocyte maturation, although only a small percentage of the original pool of immature oocytes is capable of developing to the blastocyst stage and subsequently resulting in pregnancy. In vitro maturation, as it is currently undertaken, does not support the correct development of oocyte competence. Follicle size affects oocyte quality, potentially implicating messenger RNA or protein stores as factors involved in oocyte competence. Oocytes from preantral follicles grown in vitro are competent to resume meiosis, although development to the blastocyst stage is decreased. An offspring from oocytes produced

using this technique was normal at birth but experienced delayed onset health issues, highlighting the importance of oocyte quality long after embryogenesis. Metabolism may play a critical role in oocyte quality because glycolytic activity in mature oocytes is correlated with increased embryonic development. Communication between the oocyte and its surrounding cumulus cells is also important for the development of a competent oocyte. Ovarian stimulation causes delayed embryonic development, increased abnormal blastocyst formation, fetal growth retardation, and increased fetal loss. Thus, although meiosis and even early development may be completed successfully, there are a variety of other processes occurring within the cytoplasm of the oocyte that are required for complete developmental competence. However, the cellular mechanisms that impart oocyte quality are unclear. Until the mechanisms involved in oocyte quality are elucidated, any effort to use assisted reproductive technologies in animals for production or biomedical purposes will be inefficient at best.

Key Words: Oocyte, Maturation, Meiosis, Metabolism

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Introduction

Oocyte quality impacts early embryonic survival, the establishment and maintenance of pregnancy, fetal development, and even adult disease. Quality, or developmental competence, is acquired during folliculogenesis as the oocyte grows and during the period of oocyte maturation. Assisted reproductive technologies involv-

ing ovarian hyperstimulation, or collection of immature oocytes followed by maturation in vitro, perturb this process and results in oocytes with reduced developmental competence. Although meiosis, or nuclear maturation, may be completed successfully, there are a variety of other processes occurring within the cytoplasm of the oocyte that are required for complete developmental competence following fertilization. Successful completion of these events is independent of nuclear maturation and is collectively referred to as cytoplasmic maturation. An oocyte that has not completed cytoplasmic maturation is of poor quality, and thus unable to successfully complete normal developmental processes. However, the cellular mechanisms that impart oocyte developmental competence are entirely unclear. Until the mechanisms involved in oocyte quality are elucidated, any effort to use assisted reproductive technologies in the treatment of human infertility or animal production for biomedical purposes will be inefficient at best.

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In this discussion, the components of oocyte quality and their impact on embryonic developmental potential after fertilization will be investigated. The current state of knowledge will be reviewed and data resulting from my studies will be presented in an attempt to clarify the problems and progress in oocyte quality research.

Cytoplasmic Maturation

Poor oocyte quality is the cause of infertility in a significant number of the 1.6 million American couples unable to conceive. In the United States, 15% of women of childbearing age have received an infertility treatment. There were 86,822 Assisted Reproductive Technology (ART) cycles performed in 1999, resulting in the birth of 30,285 babies (CDC/SART, 1999). Unfortunately, 69% of in vitro fertilization (IVF) cycles do not result in pregnancy. The woman's age is the most important factor affecting the chance of live birth and risk of miscarriage when her own oocytes are used. Currently, about 10% of all ART cycles (9,066 cycles in 1999) use donor oocytes. With her own oocytes, a 29-yr-old woman has about a 40% chance of achieving pregnancy during an ART cycle. This figure drops to 32% by 36 yr and nearly 0% by 46 yr. However, when donor oocytes are used the chance of achieving pregnancy during ART stays around 40% regardless of the age of the patient. Clearly, oocytes from older women are less competent. These statistics exemplify the importance of cytoplasmic maturation and oocyte quality in determining the subsequent developmental competence of oocytes.

As an oocyte grows and matures, it acquires the ability to resume and complete meiosis, successfully undergo the fertilization process, and initiate and sustain embryonic development (First et al., 1988). In the course of acquiring these competencies, cytoplasmic changes occur that may include such cellular processes as mRNA transcription (Hunter and Moor, 1987; Kastrop et al., 1991; Farin and Yang, 1992), protein translation (Sirard et al., 1989), post-translational modification of proteins (Levesque and Sirard, 1995), and ultrastructural changes (Kruip et al., 1983; Hytell et al., 1986). During growth of the oocyte within the follicle, many genes are expressed (Schultz, 1986). Some of these proteins, such as maturation promoting factor and *mos*, are involved in meiotic progression and cell cycle control (Wickramasinghe and Albertini, 1993). Other gene transcripts and their respective protein products may be involved in cellular processes critical for developmental success before and after activation of the zygotic genome (Barron et al., 1989; Watson et al., 1999). For example, blastocyst formation in the mouse is dependent on oocyte transcripts generated before fertilization (Renard et al., 1994; De Sousa et al., 1998). Identification of differentially transcribed molecules may lead to the identification of macromolecules imparting developmental competence.

Oocyte developmental competence is also affected by follicle size (Pavlok et al., 1992; Lonergan et al., 1994; Marchal et al., 2002). During the growth phase, arrested oocytes accumulate a large store of messenger RNA (mRNA) and proteins that function after fertilization to support and regulate preimplantation embryonic development (De Sousa et al., 1998). It seems that oocytes from small follicles have not yet completed cytoplasmic maturation. This may reflect deficient mRNA or protein accumulation (Pavlok et al., 1992; Pavlok et al., 1993; Lonergan et al., 1994).

Communication between the oocyte and its surrounding cumulus cells is critical for the development of a competent oocyte at ovulation. Pig oocytes clearly depend on the presence of follicle cells to generate specific cellular signals that coordinate oocyte growth and maturation (Moor and Dai, 2001). Abattoir-derived oocytes matured in medium conditioned by follicular shell pieces of Meishan pigs, a breed with low embryonic mortality, were more developmentally competent than oocytes cultured with medium conditioned by follicular shell pieces from Large White pigs, a breed with high embryonic mortality (Xu et al., 1998), demonstrating the importance of cumulus cell secretions on oocyte developmental competence. Oocyte derived proteins are required for successful ovarian folliculogenesis (Dong et al., 1996). Oocyte growth during folliculogenesis is regulated by granulosa cell derived proteins (Sterneck et al., 1997), which are in turn regulated by oocyte-derived factors (Elvin et al., 1999). Also before ovulation, oocyte secreted proteins signal somatic cells to initiate ovulation. Recent evidence suggests that both oocytes and cumulus cells produce secreted proteins and cell surface receptors that are involved in an oocyte-granulosa cell regulatory loop (Taft et al., 2002). Thus, it is imperative to examine transcription in both the oocyte and cumulus cells when investigating developmental competence.

Treatment of human infertility typically involves the administration of exogenous gonadotropins to aggressively stimulate the ovaries to produce the maximal number of mature oocytes, which are then harvested and fertilized in vitro. Ovarian stimulation in mice causes delayed embryonic development, increased abnormal blastocyst formation, fetal growth retardation, and increased fetal loss (Van der Auwera and D'Hooghe, 2001). The effects of ovarian stimulation on oocyte quality may be mediated via loss of direct interaction between the oocyte and cumulus cells (Combelles and Albertini, 2002). These negative effects are due to impaired oocyte quality, as abnormal development was initiated before the pronuclear stage (Van der Auwera and D'Hooghe, 2001). Similar detrimental effects of superovulation on oocyte quality may occur in human assisted reproduction, as embryonic development is delayed after in vitro fertilization and low birth weights are often observed. The development of oocyte quality assays employing molecular markers of developmental competence could be used to assess novel stimulation

protocols. Alternatively, oocytes could be collected without stimulation regimens during human assisted reproduction, in an immature state, and matured *in vitro*.

In humans, only a few successful pregnancies have been reported from embryos produced *in vitro* from immature oocytes (Cha et al., 1991; Barnes et al., 1995; Liu et al., 1997). In domestic livestock species, offspring have been produced using *in vitro* maturation, fertilization, and culture of oocytes (Brackett et al., 1982; Eye-stone and First, 1989; Yoshida et al., 1993b; Thompson et al., 1995; Machaty et al., 1998; Kikuchi et al., 1999). Only a small percentage of the original pool of immature oocytes placed into a maturation system is capable of developing to the blastocyst stage and subsequently resulting in pregnancy (Bavister, 1995). Similarly, early studies demonstrated that immature human oocytes are capable of initiation and completion of nuclear maturation *in vitro* and can sometimes be fertilized, although these oocytes are often not capable of supporting subsequent embryonic development (Edwards, 1965; Edwards et al., 1969; Cohen et al., 1980). This indicates that maturation of oocytes *in vitro* may not be occurring in an entirely normal manner. If immature oocytes could be successfully matured *in vitro* without prior gonadotropin stimulation, oocytes from women undergoing ovariectomy, oocyte donation programs (Cha et al., 1991) or patients with polycystic ovarian syndrome (Trounson et al., 1994) could be rescued in addition to providing more optimal treatment for infertile patients. Oocyte maturation *in vitro* is not yet efficient enough for routine clinical application. Current protocols that harvest immature oocytes from unstimulated follicles result in clinical pregnancy rates of only 2 to 13% (Cha and Chian, 1998; Trounson et al., 1998, 2001; Mikkelsen et al., 2000). Thus, although oocyte maturation *in vitro* has been accomplished, it currently results in reduced developmental potential. Unfortunately, there is no method to measure completion of cytoplasmic maturation, other than successful fertilization and embryonic and fetal development.

Oocyte Metabolism

Oocytes matured *in vitro* often have altered energy metabolism and reduced developmental potential. This may reflect a deficiency in the maturation medium, the intrinsic ability of the oocyte itself, or both. However, unless the oocyte is able to correctly control its metabolism, it will exhibit reduced viability. Regulation of nutrient metabolism is controlled at several levels, including substrate availability in the environment, transport systems in the plasma membrane, and enzyme activity and regulation (Gardner et al., 2000). These mechanisms may be critical in the oocyte to create an environment supportive of nuclear and cytoplasmic maturation. It has been well demonstrated that embryos lose the ability to regulate their metabolism correctly when cultured for even a short period *in vitro* (Thompson, 1997; Gardner, 1998; Lane, 2001), and this may be the

case for oocytes as well. Previous reports suggest that oxidative metabolism is the primary site of energy production during maturation of mammalian oocytes (Biggers et al., 1967; Brinster, 1971; Rieger and Loskutoff, 1994). However, increased glycolytic activity is associated with increased developmental competence in bovine oocytes (Krisher and Bavister, 1999). Increased glucose metabolism is a striking feature of *in vivo*-matured porcine oocytes (my unpublished data), which are known to have high developmental potential. Thus, the control of maturation and developmental capacity may be more complex than the provision of adequate energy for cellular processes.

Metabolism of glucose through the Krebs cycle and oxidative phosphorylation results in much greater energy (ATP) production than glycolysis. However, glucose metabolism may be important for the production of ribose and NADPH used in cellular processes, such as reduction of intracellular glutathione, an important antioxidant, and purine synthesis. Energy production and reactive oxygen species are closely linked. Oxidative damage results in impaired mitochondrial function, which further contributes to oxygen radical formation, reduced ATP concentration, and decreased intracellular glutathione, all of which have been associated with decreased developmental competence (Quinn and Wales, 1973; Yoshida et al., 1993a; De Matos et al., 1995, 1996; Grupen et al., 1995; Van Blerkom et al., 1995; Tarin, 1996).

Glucose metabolism is essential in the control of meiosis in the mouse oocyte. This control mechanism is not dependent on energy availability, but potentially on the generation of building blocks for purine metabolism by the pentose phosphate pathway (**PPP**) (Downs et al., 1998). Maturation induced by FSH stimulates hexokinase activity and increases glucose metabolism, resulting in high concentrations of phosphoribosyl-pyrophosphate (required for purine metabolism and formed from the end product of PPP) and germinal vesicle breakdown (Downs et al., 1998). Alternatively, glucose metabolism is tied to environmental oxygen tension and reactive oxygen species (**ROS**) concentrations within the oocyte. Excessive oxidative stress appears to contribute to reduced development of oocytes and embryos *in vitro* (Guerin et al., 2001; Orsi and Leese, 2001; Tatemoto et al., 2001). Under conditions of high oxygen during *in vitro* maturation of bovine oocytes, low glucose is necessary to maintain developmental competence. Reduced glucose concentrations resulted in decreased ROS and increased glutathione (**GSH**) concentrations. High glucose may inhibit enzymes responsible for GSH synthesis, thus impairing the oocytes ability to reduce ROS. Thus, low glucose is necessary to protect the oocyte against oxidative stress under high oxygen conditions (Hashimoto et al., 2000b). Low oxygen tension during *in vitro* maturation of bovine oocytes is beneficial for developmental competence, potentially due to decreased ROS. Although ATP production and progression to metaphase II is inhibited in a low oxygen

environment, when glucose is added to these conditions ATP concentrations and the proportion of oocytes reaching MII increases. Although ATP may be necessary for completion of nuclear maturation, under low oxygen conditions this ATP results from glycolytic metabolism of glucose. Thus, low oxygen and high glucose result in increased developmental competence in bovine oocytes by decreasing ROS and increasing ATP production via glycolysis (Hashimoto et al., 2000b). Although exceedingly high levels of ROS in the oocyte can cause oxidative damage to the cell, H_2O_2 can also function as a second messenger leading to gene expression, including some enzymes of glucose metabolism (Hashimoto et al., 2000a). In fact, a number of genes can be responsive to redox potential (Harvey et al., 2002). It may be that this function of ROS is critical to maturation success. In fact, increased ROS at the beginning of in vitro oocyte maturation improves subsequent developmental competence (Blondin et al., 1997). The follicular environment is hypoxic for the majority of the oocyte's residence and only receives the signal to increase blood flow in preparation for ovulation and luteinization at the LH surge, when maturation is reinitiated.

Studies on early embryonic development in diabetic mice have also demonstrated the importance of glucose metabolism in developmental potential. Oocytes and embryos from diabetic mice display delayed meiotic maturation and preimplantation development, an effect reversible with insulin administration (Diamond et al., 1989). This effect was duplicated when in vitro culture conditions contained elevated glucose levels (Diamond et al., 1991). Embryos cultured in high glucose concentrations display elevated, dose-related intracellular glucose concentrations, high concentrations of metabolites and developmental delays, suggesting a metabolic abnormality is involved in delayed development (Moley et al., 1996). It is interesting that high glucose culture conditions induce embryo fragmentation and modulate expression of an apoptotic gene, *Bax*, in the mouse blastocyst (Moley et al., 1998a). Specifically, high glucose culture conditions alter gene expression, triggering a decrease in facilitative glucose transporter 1 and a decrease in intracellular glucose, which acts as a cell death signal inducing apoptosis (Moley et al., 1998b, 2001; Chi et al., 2000). Hyperglycemia results in the accumulation of reactive oxygen species and mitochondrial damage within embryonic cells, resulting in ATP depletion and also triggering apoptosis (Wiznitzer et al., 1999; Moley, 2001). These studies demonstrate that high glucose levels during mouse preimplantation embryo development cause metabolic anomalies, resulting in diminished ATP stores, increased oxygen radicals, and altered gene expression, leading to apoptosis and potential malformations in the resulting fetus. Exposure of oocytes to elevated glucose concentrations during the maturation period may cause similar deleterious effects in the subsequent embryo.

From our experimental results (our unpublished data), we know that glucose metabolism through glycol-

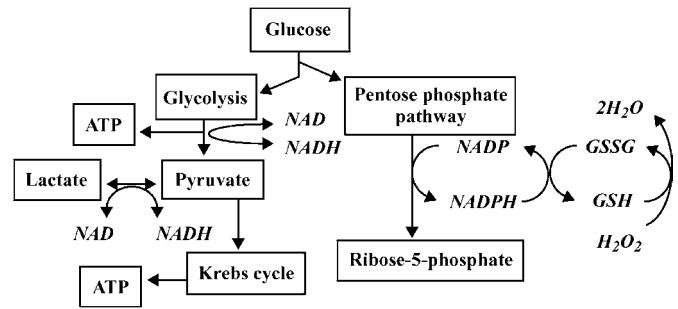


Figure 1. Relationship between glucose metabolism and redox potential, including metabolic effects on ATP, glutathione, and reactive oxygen species concentration within the cell. Molecules affecting redox potential are italicized. GSH = reduced glutathione, GSSG = oxidized glutathione.

ysis and the PPP is significantly higher in oocytes matured in vivo. Specifically, the activity of PPP is very high in mature porcine oocytes, a characteristic unique, as far as we know, to this species. This information has led us to believe that glucose metabolism, and particularly the pentose phosphate pathway, are involved in the normal mechanisms regulating the resumption of meiosis and maintenance of developmental competence. The mechanisms, however, remain unknown, and there are several possibilities. End products of glucose metabolism may be important to meiotic resumption, as has been suggested in mice (Downs et al., 1998). There may be a requirement for ATP resulting from metabolism, as has been suggested for cattle (Hashimoto et al., 2000a). Glutathione and reactive oxygen species may also play a role (Hashimoto et al., 2000b). One common thread unifying all of these hypotheses is the redox potential of the cell (Figure 1). Glucose metabolism via glycolysis results in limited ATP production itself, but the end products, pyruvate and lactate, can be metabolized in the Krebs cycle to produce high quantities of ATP under aerobic conditions. Pyruvate can also be reduced to form lactate, thus altering the NAD:NADH ratio and the redox potential. The PPP offers even more opportunities for the cell to control redox potential. The pathway itself requires NADP and produces NADPH. In addition, glutathione recycling can occur, changing glutathione from its reduced to oxidized form as it reduces ROS within the cell and then back to the reduced form using NADPH. All of these molecules contribute to the redox potential of the cell. Nuclear and cytoplasmic maturation may be controlled not by energy requirements (ATP), building blocks (ribose-5-phosphate), or oxidative stress (ROS) but by the redox potential within the oocyte (Figure 1). Measurements of glutathione, ATP or ROS are simply indicative of this state. A similar hypothesis has recently been proposed for developmental capacity of mammalian preimplantation embryos (Harvey et al., 2002).

Further evidence of metabolic influence on oocyte developmental competence arises from studies of oocyte growth from the primordial follicle to meiotic maturation completely *in vitro* in mice (Eppig and O'Brien, 1995). During culture of oocyte-granulosa cell complexes, FSH and insulin stimulate abnormal LH receptor expression in the surrounding granulosa cells. Although competence to resume meiosis was not affected in these oocytes developmental potential was reduced, demonstrating the deleterious effects of inappropriate oocyte culture conditions (Eppig et al., 1998). Oocytes from preantral follicles grown *in vitro* have resulted in successful preimplantation development and birth of a single offspring (Eppig and O'Brien, 1996, 1998). Although normal at birth the mouse developed severe health problems with age, suggesting that suboptimal *in vitro* growth and development of oocytes may be contributed to delayed onset health issues (Eppig and O'Brien, 1998).

Ions and Mitochondria

Transient increases in intracellular Ca^{2+} concentration may spontaneously activate and regulate the meiotic and cytoplasmic maturation of the oocyte. Whereas many studies have focused on Ca^{2+} oscillations during the fertilization of the mature oocyte, few studies have examined intracellular Ca^{2+} levels within the immature oocyte (prior to arrest at metaphase II). In immature mouse oocytes, Ca^{2+} transients occur every 1 to 3 min following the release of the oocyte from the ovarian follicle and persist for approximately 1 to 3 h (Carroll et al., 1994). Yet, it remains controversial whether Ca^{2+} transients in immature mouse oocytes actually influence germinal vesicle breakdown (GVBD). In bovine oocytes, chelation of intracellular Ca^{2+} results in the blockage of spontaneous GVBD (Homa, 1995). Porcine oocytes cultured in a Ca^{2+} -free medium in the presence of a Ca^{2+} chelator, experience a Ca^{2+} transient before the oocyte undergoes GVBD (Kaufman, 1993). This suggests that an increase in intracellular Ca^{2+} triggers GVBD in pig oocytes.

The source of the Ca^{2+} required during oocyte maturation is also of significant interest. Certain mammalian oocytes, including porcine, matured in a Ca^{2+} -free environment, apparently rely only on intracellular sources of Ca^{2+} , like endoplasmic reticula and mitochondria, to initiate GVBD (Paleos and Powers, 1981; Kaufman, 1993). Recently inositol 1,4,5-trisphosphate (IP_3) and ryanodine release channel receptors were found to be located on the surface of the endoplasmic reticulum within porcine oocytes. Interestingly, these receptors are active from the germinal vesicle stage throughout maturation to metaphase II and possibly even to interphase (Machaty, 1997), enabling increased amounts of Ca^{2+} to be released from the intracellular stores. Whereas GVBD in pigs may depend on internal stores of Ca^{2+} , the continuation of meiosis after metaphase I and polar body formation may require extracellular

Ca^{2+} (Kaufman and Homa, 1993; Homa, 1995), provided by the reproductive tract (*in vivo*) or the culture medium (*in vitro*).

Elevated intracellular Ca^{2+} levels, the result of cellular stress or high extracellular Ca^{2+} concentrations, may perturb normal maturation. Under physiological conditions, Ca^{2+} transients serve as second messengers in mammalian cells (Campbell, 1983) and stimulate mitochondrial oxidative metabolism and ATP production. Under pathological conditions, a prolonged increase in intracellular Ca^{2+} , may activate destructive enzymatic pathways and inhibit energy producing systems the cell (Beitner, 1993). Excess Ca^{2+} directs the opening of the mitochondrial permeability transition pore, or megachannel (Krieger, 2002), resulting in the release of cytochrome C and the activation of proteolytic caspases that lead to apoptotic cell death. A disrupted mitochondrial membrane also diminishes the mitochondrial membrane potential. Without a functional mechanism to produce energy, the oocyte will undergo necrotic cell death upon consumption and depletion of energy stores. Cytosolic magnesium (Mg^{2+}) inhibits the opening of this megachannel. As a counter ion to cellular Ca^{2+} , Mg^{2+} has the ability to lower intracellular Ca^{2+} concentrations and therefore regulate and alleviate the adverse effects of elevated Ca^{2+} (Altura et al., 1982). In addition to Ca^{2+} , energy in the form of ATP is critical for nuclear and cytoplasmic maturation events. Because mitochondria synthesize ATP and serve as an internal source of Ca^{2+} , it is possible that mitochondria redistribute and aggregate within the oocyte to concentrate ATP (Ducibella et al., 1977) and Ca^{2+} (Mehlman et al., 1996; Li, 1997) at sites of high demand. During oocyte maturation, breakdown of the nuclear membrane likely requires elevated ATP levels. Determining the distribution and activity of mitochondria in response to a changing ionic environment may be key to understanding the role of mitochondria during the maturation of porcine oocytes.

Experimental Approach

To understand cytoplasmic maturation of mammalian oocytes in relation to developmental potential, my laboratory has taken the approach of comparing oocytes with high and low developmental potentials. By comparing two populations of oocytes, for example *in vivo* and *in vitro*-matured or large- and small follicle-derived, the differences discovered may indicate important mechanisms involved in developmental competence. In this way, future research may be focused and important mechanisms understood more rapidly. Ultimately, the best test of oocyte quality is the transfer of embryos produced from known oocytes, followed by gestation, birth, and continuing health of the resulting offspring. However, these experiments are cost prohibitive, take a great deal of time to achieve results, and require large animal numbers to obtain data that can be statistically analyzed. Thus, other parameters can

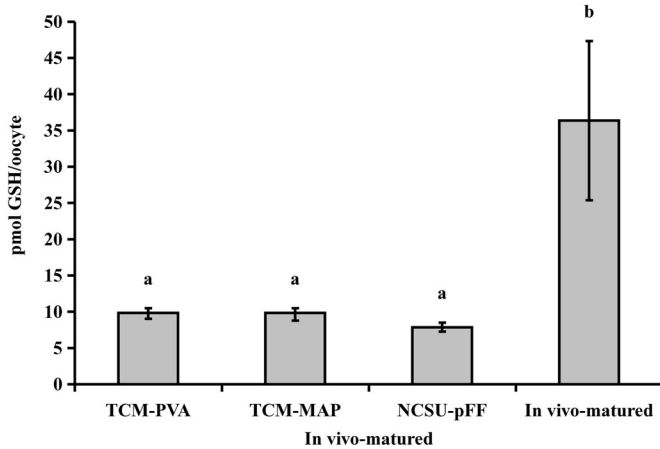


Figure 2. Intracellular glutathione concentration of *in vivo* and *in vitro*-matured porcine oocytes. Oocytes were matured *in vitro* in one of three maturation media; tissue culture medium-199 with polyvinyl alcohol (TCM-PVA), TCM-199 with hyaluronic acid (trade name MAP5; TCM-MAP), or North Carolina State University-23 with porcine follicular fluid (NCSU-pFF). ^{a,b}Different superscripts represent statistical differences between treatments ($P < 0.05$). Data are presented as mean \pm SEM. From Brad et al. (2003).

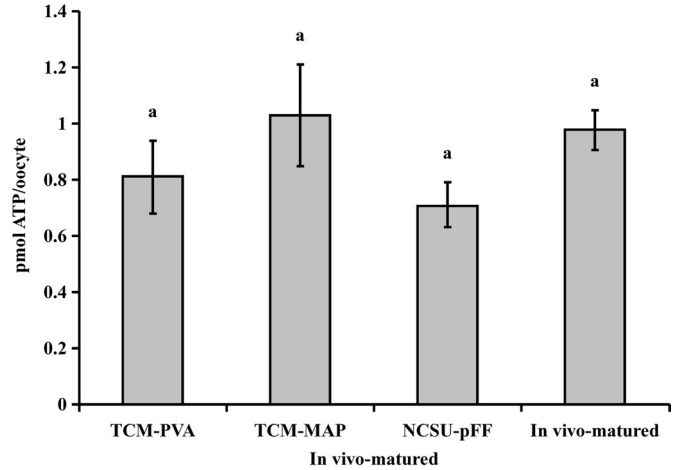


Figure 3. Concentration of ATP in porcine oocytes matured *in vivo* and *in vitro*. Oocytes were matured *in vitro* in one of three maturation media; tissue culture medium-199 with polyvinyl alcohol (TCM-PVA), TCM-199 with hyaluronic acid (trade name MAP5; TCM-MAP), or North Carolina State University-23 with porcine follicular fluid (NCSU-pFF). ^{a,b}Different superscripts represent statistical differences ($P < 0.05$). Data are presented as mean \pm SEM. From Brad et al. (2003).

be used to help assess viability. Endpoints that we have used for this purpose include nuclear maturation success (to metaphase II), fertilization (normal with two pronuclei), development to the blastocyst stage, blastocyst total cell number, inner cell mass and trophectoderm cell numbers, oocyte metabolism, metabolism of the subsequent embryo, metabolic pathway preferences, ATP and ADP content, glutathione content, mRNA and proteins present, and mitochondrial activity and distribution. Examination of all of these parameters will assist in discovering the complex, multifaceted mechanisms that function synchronously to impart oocyte quality.

Much of our experiment work has dealt with metabolic variables and how they relate to developmental competence in oocytes. Metabolic activity of *in vivo*-matured porcine oocytes was determined and compared to metabolism of porcine oocytes matured under standard *in vitro* maturation conditions (Durkin et al., 2001). Our findings indicate *in vivo*-maturing porcine oocytes use glucose via glycolysis as their primary energy substrate. Glucose is metabolized equally via the PPP and the glycolytic pathway in *in vivo*-matured oocytes. *In vitro*-matured porcine oocytes utilized glucose via the glycolytic pathway preferentially over all other substrates examined. Oocytes matured *in vitro* utilize significantly less glucose via PPP than *in vivo*-matured oocytes. Overall, *in vitro*-matured oocytes were less metabolically active than those matured *in vivo*. Interestingly, when concentrations of glucose, pyruvate, and lactate similar to what we found in follicular fluid were included in maturation medium, the metabolic re-

sponse of the embryos was most like that found in *in vivo*-matured oocytes (Brad et al., 2003b). Although the concentration of energy substrates was lower than what is commonly found in maturation media, the metabolic activity of the oocytes was higher.

In addition, we have compared GSH and ATP concentrations of *in vivo* and *in vitro*-matured porcine oocytes (Brad et al., 2003a). Oocytes matured *in vitro* in defined tissue culture medium-199 (TCM-199) with polyvinyl alcohol, TCM-199 with hyaluronic acid (trade name MAP5) or North Carolina State University-23 with porcine follicular fluid had significantly lower concentrations ($P < 0.05$) of GSH compared with *in vivo*-matured oocytes (Figure 2). Concentrations of ATP were not different between treatments (Figure 3). Thus, concentration of intracellular ATP content does not appear to be related to developmental potential in porcine oocytes. Low intracellular GSH may be related to or responsible for lower developmental competence observed in *in vitro*-matured porcine oocytes. We have also attempted to manipulate glucose metabolism using various chemical inhibitors and stimulators in order to assess the interactions between metabolism, intracellular glutathione content, nuclear maturation, and embryonic development. Cumulus-oocyte complexes were matured in the absence of chemicals (control) or in the presence of a PPP stimulator (pyrroline carboxylic acid) or a PPP inhibitor (diphenyleneiodonium). Inhibition of the PPP significantly increased the percentage of immature and reduced the percentage of mature oocytes, decreased metabolism, inhibited embryonic development, and lowered GSH concentrations (Herrick et al., 2003). In-

terestingly, regression analysis of end point parameters in control oocytes demonstrated that glycolytic activity was significantly correlated with progression to metaphase II. The observed effects on nuclear maturation, embryonic development, and GSH suggest that glucose metabolism plays a key role in the mechanisms controlling nuclear and cytoplasmic maturation of porcine oocytes. We have also used nuclear transfer as a tool to examine cytoplasmic maturation. When recipient oocytes were treated with a PPP stimulator, more resulting embryos reached the morula and blastocyst stages and those embryos had greater Krebs cycle activity when compared to controls (Clifford et al., 2003).

Finally, we have examined the role of ionic composition of the maturation medium on mitochondrial distribution and oocyte competence (our unpublished results). At the germinal vesicle stage, mitochondria appear clustered about the germinal vesicle. After 20 h of *in vitro* maturation, mitochondrial distribution depended on ionic composition of the medium, although after 40 h, the majority of oocytes in all treatments had mitochondria clustered toward the center of the oocyte with no mitochondria in the periphery. Because ionic composition also affected development to the blastocyst stage but not metabolism, the movement of mitochondria during maturation and possibly transient metabolism may be important to developmental competence.

Future Directions

Functional genomics and proteomics are tools that promise to provide many leads into important mechanisms of oocyte competence. Functional genomics is the genome-wide monitoring of gene expression. This approach has launched the concept of the transcriptome as the identification and quantification of all the expressed genes in a population of cells or a given tissue. Monitoring changes in gene transcription on a genome-wide basis allows identification of groups or clusters of genes that are functionally related to a cell or tissue phenotype. The complementary DNA (cDNA) microarray (Schena et al., 1995) and serial analysis of gene expression are two methodologies that have matured into powerful technologies for analysis of transcriptomes. The cDNA microarray is a closed system that requires previous isolation for a cDNA to be included in a transcript profile. However, microarrays provide an economical and practical system for monitoring global changes in gene expression in large numbers of samples. Serial analysis of gene expression is an open system (Velculescu et al., 1995; Green et al., 2001), based on direct sequence determination of the number and abundance of the transcripts present in a sample, and does not require previous cDNA discovery to generate the sequence tags.

In vitro-matured human and bovine oocytes have been shown to have reduced protein content compared to *in vivo*-matured oocytes (Kastrop et al., 1991; Trounson et al., 2001), suggesting that proteins play a critical

role in acquisition of developmental competence. Proteomics is a complementary technology to DNA microarrays for monitoring gene expression. Because proteins are the functional molecules in a cell, they reflect functional differences in gene transcription, translation, and post-translational modifications. In addition to protein synthesis (Kubelka et al., 1988; Wu et al., 1996), stage-specific changes in protein phosphorylation occur during oocyte maturation (Endo et al., 1986). Two-dimensional electrophoresis provides a comprehensive and quantitative analysis of cellular proteins. Importantly, post-translational modifications can also be analyzed. After separation, proteins can be cut out of the gel, digested and subjected to mass spectrophotometry, resulting in protein identification by comparison to existing databases. Two-dimensional electrophoresis is a powerful approach, and the method of choice as it can be used to visualize a large number of proteins in a differential display format (Haynes and Yates III, 2000). This multifaceted approach gives the greatest probability of finding developmentally important proteins. This technique is particularly important because it has been suggested that transcription profiles and actual cellular protein content sometimes lack correlation (Lopez, 2000). As more and more sequence information for many of our domestic animal models becomes known, proteomics and genomics become even more powerful tools to help answer many of these outstanding questions in the investigation of oocyte quality.

Implications

Ultimately, we hope to identify biochemical markers in oocytes that are correlated with developmental competence. With this information, we can design appropriate ovarian stimulation regimens and maturation conditions, as well as assess developmental competence of oocytes matured *in vitro*. We can apply this information to both alleviate infertility and increase efficiency of *in vitro* embryo production. Research investigating the components of oocyte quality is also critical because it will enable the development of assays to test for oocyte developmental competence, improved media formulation for *in vitro* oocyte maturation, and perhaps even the identification and treatment of specific types of infertility by RNA or protein injection into oocytes. In addition, the production of competent oocytes will positively affect the application of nuclear transfer and transgenic technologies to animals for agricultural applications, as well as the production of human pharmaceutical proteins.

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