BRIEF COMMUNICATION

Impact of oocyte membrane integrity on survival of oocytes post-ICSI and generation of viable embryos

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Abstract

Objective
The objective of this study is to determine the impact of membrane quality on survival and viability of oocytes post-ICSI.
Materials and Methods
(1) Mature oocyte (MO; defined as healthy oolemma that offers resistance to the ICSI needle), (2) Partially post-mature oocyte (PPM; offers partial resistance and (3) post-mature oocyte (PM; defined as unhealthy oolemma that offers no resistance). Embryos derived from MO oocytes are prioritized for embryo transfer. Embryos from PPM and PM derived oocytes will not be transferred except when MO-derived embryos are not available, or insufficient, or were of extreme poor quality.
Results
There were significant differences between MO, PPM and PM oocytes with regards to fertilization [90.1% (109/121); 65.9% (29/44); 22.4% (13/58), p<0.0005 respectively], and degeneration of oocytes post-ICSI [0% (0/121); 13.6% (6/44); 67.2% (39/58), p<0.0001 respectively], with superior characteristics demonstrated only by the MO, followed by the PPM and PM oocytes in decreasing order. A similar scenario exist for embryo transfer and embryo cryopreservation rates. The combined unfertilized oocyte and unutilized embryo (%) rates were significantly low in the MO oocytes [41.3% (50/121); 63.6% (28/44); 94.8% (1/58), p<0.02 respectively] but is higher in PPM and PM oocytes in increasing order.
Discussion and Conclusion
The evidence points to the critical importance of oolemma integrity for the generation of viable embryos. It is obvious that MO oocytes are superior to PPM and PM oocytes, with PM being most inferior in quality. The reasons for poor outcome in PPM and PM may be due to oocyte post-maturity, difficult ovarian stimulation and inherently abnormal oocytes of variable causes. In conclusion a high proportion of pregnancies could be obtained from MO oocytes but not from PPM and PM oocytes indicating oocytes that offer no or diminished resistance to the ICSI needle are aged and should not be utilized. It is documented that embryos derived from aged oocyte carry health risks to the conceptus.

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Introduction

It is felt that 5–25% of conventional IVF treatment cycles fail due to total fertilization failure (TFF; Barlow et al., 1990; Chen et al., 1995). Initially, the ART community resorted to rescue ICSI (r-ICSI) at about 18-24hrs post-ICSI (Batha et al., 2023; Esfandiari et al., 2008; Morton, 1997; Singh et al., 2013) following TFF after conventional IVF (cIVF) to prevent treatment failure with some measure of success but others have noted poor treatment outcome (Kuczynski et al., 2002; Chen and Kattera 2003; Dozortsev et al., 2004; Amarin et al., 2005; Ming et al., 2012) or in some case it was reported that such oocytes are genetically damaged (Nagy et al., 1995). Another strategy to avoid TFF and oocyte aging is to perform r-ICSI earlier, usually at about 4-6 hours post insemination (Chen and Kattera, 1995; Nagy et al., 2006; Wei et al., 2011; Xue 2013; Liu et al., 2014; Zhou et al., 2016; He et al., 2018). It is long well established that in stimulated cycles there is a asynchrony in the maturity of oocytes within the same cohort of oocytes (Sundstrom et al., 1988; Hammitt et al., 1993), such that while most oocytes are mature, but some may be immature and post-mature.

During r-ICSI in TFF oftentimes the oolemma of some of the 24-hours old but not the 4 to 6-hours oocytes offered no resistance to the ICSI needle. A significant proportion of such oocytes failed to survive after ICSI (JA, Unpublished observations) degenerating almost immediately after needle penetration or subsequently. Whereas those that offered resistance to the ICSI needle almost always survived.

This led us to assume that the viability of oocytes that offered no resistance to the ICSI needle are somehow compromised either due to aging (post-mature?) or due to some form of damage or environmental insult. Indeed about 10% of r-ICSI oocytes degenerated (Morton et al.,1997). Patients on warfarin had oocytes with fragile membranes (Ali, Unpublished observations). It is well recognized that it is quite common in any cohort of oocytes in most patients to often have one or more oocytes with oolemma that offer no resistance to the ICSI needle.

Since it is well recognized oocytes retrieved in stimulated cycles are asynchronous in maturity (Sundström and Nilsson, 1988; Hammitt et al., 1993; Ali et al., 1998) we asked the questions what is the viability rate of oocytes that demonstrate varying levels of resistance to the ICSI needle and what proportion of these ICSI-ed oocytes fertilized, degenerated, were transferred, cryopreserved and what was the pregnancy rate per embryo transfer? The objective of the present study is to determine the impact of membrane quality on the developmental potential of the oocytes post-ICSI with respect to the degree of oolemma integrity or resistance to ICSI.

Materials and methods

Oocytes with normal morphology with oolemma that offered resistance to the ICSI needle were considered mature oocytes (MO), those oocytes with oolemma that exhibited partial resistance were assumed to be partially post mature (PPM), while oocytes with oolemma that offered no resistance at all to the ICSI needle were assumed to be post-mature (PM). Oolemma that offered no resistance to the ICSI needle is assumed unhealthy possibly due to aging apopotosis. Embryos derived from MO oocytes are prioritized for embryo transfer. Embryos from PPM and PM derived oocytes will not be transferred except when quality MO-derived embryos are not available, or insufficient, or are of extreme poor quality.

Results

There were significant differences between MO, PPM and PM oocytes with regards to fertilization [90.1% (109/121); 65.9% (29/44); 22.4% (13/58), $p<0.0005$ respectively], and degeneration of oocytes post-ICSI [0% (0/121); 13.6% (6/44); 67.2% (39/58), $p<0.0001$ respectively], with superior characteristics demonstrated only by the MO, followed by the PPM and PM oocytes in decreasing order. A similar scenario exists for embryo transfer and embryo cryopreservation rates (Table 1). The proportion of combined unfertilized oocyte and unutilized embryo rates were significantly low in the MO oocytes but higher in PPM and PM oocytes [41.3% (50/121); 63.6% (28/44); 94.8% (1/58), $p<0.02$ respectively] in increasing order.

Discussion

The findings of this study pose the question whether the oocytes with compromised oolemma integrity are useful in ART treatment? The findings of this study clearly shows the poor outcome with all parameters tested, such as post-ICSI, oocyte degeneration rate, fertilization

Table 1: Characteristics of fertilization, survival post-icsi and survival of embryos derived from oocytes of variable quality.

Oocyte Membrane Quality	Fertilization/ Total Oocytes (%)	Oocytes that degenerated after ICSI	Oocytes that survived ICSI but did not fertilize (%)	Total unfertilized oocytes and unutilized embryos (%)	Embryo Transfer (ET) (%)	Frozen Embryos (blastocyst) (%)	Pregnancy/ Total patient for ET (%)
M	n= 109/121 90.1%	n= 0/121 0%	n= 12/121 9.9%	n= 50/121 41.3%	n= 44/121 36.4%	n= 27/121 22.3%	n= 13/28 46%
PPM	n= 29/44 65.9%	n= 6/44 13.6%	n= 9/44 20.5%	n= 28/44 63.6%	n= 10/44 22.7%	n=6/44 13.6%	n= 1/7 14.3%
PM	n= 13/58 22.4%	n= 39/58 67.2%	n= 6/58 10.3%	n= 55/58 94.8%	n= 1/58 1.72%	n= 2/58 3.44%	n= 0/1 0

Table 2 Statistical comparison between: MO vs PPM; MO vs PM; PPM vs PM

Oocyte Membrane Quality	Fertilization/ Total Oocytes (%)	Oocytes that died after ICSI	Oocytes that survived ICSI but did not fertilize (%)	Total unfertilized oocytes and unutilized embryos (%)	Embryo Transfer (ET) (%)	Frozen Embryos (blastocyst) (%)	Pregnancy/ Total patient for ET (%)
MO VS PPM	p = 0.0005	p = 0.0003	p = 0.1229	p = 0.0182	p = 0.1414	p = 0.3097	N.A
MO VS PM	p < 0.0001	p < 0.0001	p = 0.8552	p < 0.0001	p < 0.0001	p = 0.0028	N.A
PPM VS PM	p < 0.0001	p < 0.0001	p = 0.2467	p = 0.0002	p = 0.0022	p = 0.1284	N.A

rate, proportion of embryos available for ET, the level of embryo arrest and demise rates, proportion of embryos available for cryo-storage and pregnancy rates were severely affected or compromised in PPM and PM oocytes. The limitation of this study is its small sample size. A larger study is needed to corroborate conclusively the findings of the present work. Nevertheless the inferiority of oocytes with compromised membrane integrity was clearly demonstrated, clearly discernible and is readily apparent.

It is not clear what causes poor oolemma integrity. Based on our observations on r-ICSI in 6- and 24-hours old r-ICSI procedures, we are tempted to speculate the lack of membrane integrity is due to oocyte aging, because the 6-hour r-ICSI operation do appear to encounter no or very few oocytes with compromised oolemma integrity. This lends credence to our assumption that the poor membrane integrity could be a manifestation of apoptosis related to aging of the oocyte. This suggestion remains to be elucidated conclusively. Other factors that appear to contribute to poor oolemma integrity are poor ovarian response to stimulation or inherent membrane abnormality, and medication but these remains to proven conclusively.

Oftentimes the rescue of failed fertilization cases involves the use of 24-hour in-vitro aged oocytes. It is not appropriate 24-hour aged oocytes be used to treat patients (Coticchio, 2013). The authors correlate the observation of oocytes with poor oolemma integrity in 24-hour but not 6-hour old r-ICSI oocytes to oocyte aging. In view of this the authors content that freshly retrieved oocytes with diminished or deficient oolemma integrity probably are aged because of the fact the maturity of oocytes in stimulated cycles are asynchronous (Sundström and Nilsson, 1988; Hammitt et al., 1993; Ali et al., 1998) and therefore some oocytes are likely to be post-mature at time of retrieval. In both animals and humans, the use of aged oocytes carries risk to the health of the conceptus, may contribute to a decline in implantation rates, genetic abnormalities as well as biochemical and cellular degeneration (Nagy et al., 1995; Tarın et al., 1999; Tarın et al., 2002; Coticchio, 2013).

This would indicate that there is a definite need for caution if there is a link between oocyte aging and diminished oolemma integrity. It follows that embryos generated from aged oocytes must not be transferred if embryos generated from MO are available.

Conclusion

High proportion of pregnancies could be obtained from MO oocytes but not from PPM and PM oocytes. The available evidence points to the critical importance of oolemma integrity for the generation of viable embryos. This observation has policy implications. MO oocytes are superior to PPM and PM oocytes, with PM being most inferior in quality. The reasons for poor outcome in PPM and PM may be due to oocyte apoptosis related to post-maturity. Other probable causes are poor ovarian stimulation and inherently abnormal oocytes of variable causes.

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