Brief Communication

Generation of blastocyst from a zona-free oocyte.

NH Al Harbi, MY Al Raawi, LN Al Turaiki, SA Aldakhilallah

Assisted Reproductive Technology Laboratory Unit, Women's Specialized Hospital, King Fahad Medical City, Riyadh, Saudi Arabia

Abstract

Introduction
Oftentimes the laboratory IVF Practitioner is confronted with zona-free oocytes (ZFOs). Fertilizing a ZFO can be a challenge. We describe here our experience with the fertilization of a ZFO by ICSI and its subsequent development to the blastocyst stage.
Methods
Seventeen (n=17) oocytes were retrieved. 14 oocytes were normal with intact zona (ZIOs), 1xZFO and 2 x abnormal. The 14 ZIOs were inseminated by standard ICSI procedure while the ZFO was inseminated by a modified ICSI method. Briefly, the ZFO is held by a holding pipette with minimal suction pressure. An immobilized sperm is injected into the ZFO to a length of only about 1/12th of its diameter. The tip of the injection pipette penetrates to a depth just barely under the oolemma. The oolemma is compromised by gentle suction with the injection pipette and the immobilized sperm is expelled into the cytoplasm.
Results
10 of 14 ZIOs and the single ZFO were fertilized. The zygotes derived from ZIOs were cultured in groups of 5 under ultra-micro drop cultures (cUMD). Likewise, the ZFE-derived zygote was cultured singly. On day 2 three (2 small and 1 large) non-fragmented blastomeres were note from to have developed from the fertilized ZFO. On day 3 the ZFE-derived embryo developed to the 7-cell embryo stage with no fragments. On day 4 it compacted, on day 5 it had blastulated and on days 6 to 7, it expanded.
Conclusion
It may be necessary to utilize the ZFOs if the number of oocytes available to the patient is limited or if only ZFOs are available. Fertilizing a ZFO is challenging, it is very fragile and may succumb to manipulation. The question is whether the ZFO-derived embryos are normal and safe for transfer. Are the genetic constitution of the ZFO and the resultant embryo preserved intact following ICSI? The use of a polscope may enable ICSI of the ZFOs without damaging the spindle. Preimplantation genetic analysis may reveal whether the ZFO-derived embryo is genetically normal if there is a need to transfer ZFO-derived blastocysts, especially in the absence of embryos that are derived from zona-intact oocytes.

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Correspondence: Naif H Al Harbi; email: nhaalharbi@kfmc.med.sa
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Introduction

Oftentimes the laboratory IVF Practitioner is confronted with zona-free oocytes (ZFOs). When a patient has many eggs and has sufficient zona intact oocytes (ZIOs) there is no concern but should the patient has only ZFOs or very few ZIOs the presence of one or more ZFOs constitutes a loss to the patient and the treatment cycle.

There are several reports on the fertilization of ZFOs usually by ICSI with survival and development of the ZFOs to all stages of the zona-free embryos (ZFEs) including blastocysts (ZFBs; Ding et al., 1999; Bodri et al., 2015) or

degeneration of the oocytes or embryos (Stanger et al., 2001), including transfers of both fresh and vitrified ZFEs/ZFBs, that has resulted in pregnancy after cleavage stage transfer but no live-births, (Stanger et al., 2001; Hsieh et al. 2001), and live-births from both fresh and vitrified ZFEs/ZFBs (Shu et al., 2010; Ueno et al., 2014; Hu et al., 2016), abortion (Shu et al., 2010; or no pregnancies (Ding et al., 1999; Takahashi et al., Hsieh et al. 2001). Ueno et al., (2014) utilized a polscope to visualize and avoid damage to the spindle of the ZFOs during the ICSI procedure.

Subject and methods

Seventeen (n=17) eggs were retrieved. After stripping off the corona-cumulus cells, 14 were ZIOs, 1 ZFO and 2 were morphologically abnormal. The 14 ZIOs were inseminated by standard ICSI procedure while the ZFO was inseminated by a modified ICSI method. Briefly, the ZFO is held by a holding pipette with minimal suction pressure. The injection pipette containing the immobilized sperm is injected into the ZFO to a length of only about 1/12th of its diameter. The tip of the injection pipette penetrates to a depth just barely under the oolemma. The oolemma is compromised by gentle suction with the injection pipette and the immobilized sperm is expelled into the cytoplasm.

Results

10 of 14 ZIOs and the single ZFO were noted to be fertilized at about 21hrs post-ICSI. The zygotes derived from ZIOs were cultured in groups of 5 under ultra-micro drop cultures as previously described by Ali (Ali, 2004; Ali et al., 2000). Likewise, the ZFE-derived zygote was cultured singly. On day 2 three (2 small and 1 large) non-fragmented blastomeres were noted, on day 3 the ZFE-derived embryo developed to the 7-cell embryo stage with no fragments. On day 4 it compacted, 5 it blastulated and on days 6, it expanded (Figs 1-6 respectively).

Discussion

In our ICSI method for ZFOs, we have avoided deep penetration normally utilized for ICSI of ZIOs, therefore it is likely the spindles of the ZFO were not damaged but this is merely speculative. Unless a polscope was used for the ICSI procedure the operator will be oblivious of the effect of ICSI on the spindle of the ZFO. Furthermore, it is difficult to ascertain complete certainty that the ICSI procedure did not damage the spindle and consequently, the genetic status of the resultant embryo was suspect. This is because of the lack of appropriate material resources that could ensure the spindle which could be avoided during ICSI and access to preimplantation genetic screening of the blastocyst generated from the ZFO. These two techniques would have permitted the generation or the selection of genetically normal ZFEs/ZFBs respectively.

In the present case study, the development of the ZFO-derived blastocyst appears normal. The odd number of blastomeres noted on day 2 is compatible with normal development since one of the blastomeres was larger than the other two, a probable indication that the larger blastomere will cleave subsequently as is the case in normal development. Prolonged time taken for cleavage from the 2-cell to the 3-cell stage can be abnormal (Holm et al., 1998). This has been observed in time-lapse technology but without any apparent correlation to embryo development potential (Pribenzsky et al., 2010). Meseguer and coworkers (Meseguer et al., 2011) noted the duration to the 3-cell stage to be the parameter that had a significant bearing between implanting and non-implanting embryos. Embryos carrying unbalanced translocations cleaved less synchronously and were delayed in time of cleavage to the 4-cell stage (t4) and in time of start of blastulation (tSB) compared with balanced embryos (Amir et al., 2019).

We have refrained from transferring the ZFO-derived blastocyst simply because we have no clue as to whether the zona-free blastocyst is normal. The need to transfer ZFO-derived blastocyst did not occur since we had sufficient ZIO-derived embryos for transfer. Although there are reports on ZFO-derived embryos we need a larger data set on the safety of using ZFO-derived embryos before such embryos can be utilized for therapeutic purposes. The present work was performed without a time-lapse imaging facility.

The occurrence of 100% ZFOs is rare but when confronted with oocytes with this defect which are probably congenital, ICSI appears to be the only option (Stanger et al., 2001; Hu et al.,

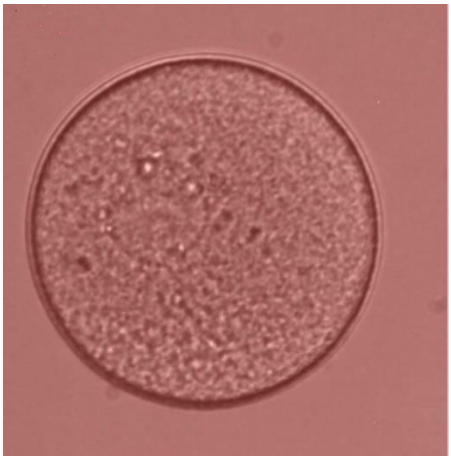


Fig. 1: Zona-free egg

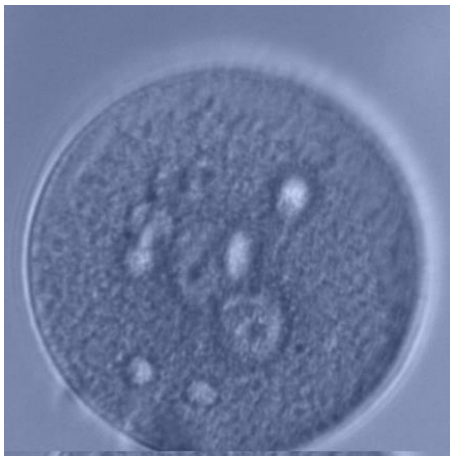


Fig. 2: 2PN pronuclear stage zona-free zygote

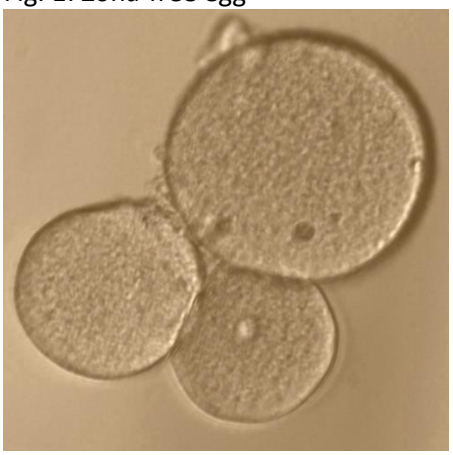


Fig. 3: Day 2 3-cell zona-free embryo

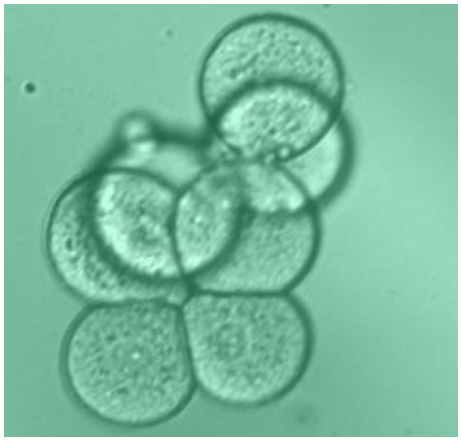
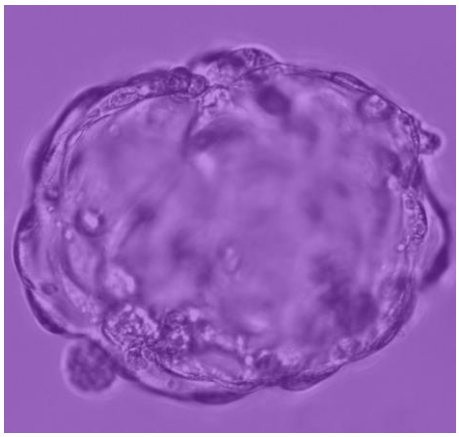


Fig. 4: Day 3 nucleated 7-cell zona-free egg

Fig. 5: Day 5 zona-free blastocyst



Day 6 Expanding zona-free blastocyst

Figs 1-6: The development of a zona-free blastocyst from zona-free oocyte after ICSI

2016; Metwally et al.,2020). In these patients, the congenital absence of the zona pellucida may be the cause of their infertility (Metwally et al., 2020) because their ZFO may be damaged by polyspermy in its natural milieu or if it was fertilized by a single sperm the blastomeres of the resultant embryo are most likely dispersed during the cleavage stages of embryo development due to the natural contractions and the dynamic action of the cilia lining the inner walls of the Fallopian tube. Therefore, the opportunity for natural pregnancy in these patients is limited. In such patients, ICSI of ZFOs may be the only option to achieve parenthood.

When the number of oocytes recovered from a patient is sufficient there is no need to utilize the ZFOs. It may however be imperative to utilize the ZFOs if the number of oocytes available to the patient is limited or if only ZFOs are available. ZFOs are very fragile and may succumb to manipulation. Often the ZFO may be sucked into the holding pipette and damaged if the operator is not careful. It is therefore necessary to devise a technique that will not harm the ZFOs during ICSI. There appears to be a need to formulate policies on the use of ZFOs. We suggest caution in the transfer of ZFEs/ZFBs and question its validity and safety when the polscope and preimplantation genetic analyses are not accessible. The birth of apparently healthy babies from ZFEs/ZFBs generated from ICSI of ZFOs without the aid of the polscope and genetic analysis may not guarantee a healthy adult with neuromotor, cognizance, metabolic, and other health conditions that could occur due to damage to the genetic constitution of the ZFO-derived embryos or blastocysts.

Conclusion

Question remains whether the genetic constitution of the ZFO and the resultant embryo preserved intact following ICSI? The use of a polscope may enable ICSI of the ZFOs without damaging the spindle. While preimplantation genetic analysis may reveal whether the ZFO-derived embryo is genetically normal if there is a need to transfer ZFO-derived blastocysts, especially in the absence of embryos that are derived from zona-intact oocytes.

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