BRIEF COMMUNICATION

Rescue of immature oocytes by combined in vitro maturation and conventional in vitro fertilization

Siti Khadijah Idris, Nuguelis Razali, Sharifah Mahfudzah, Hasnidar A. Tarmizi, Syairah Hanafiah, Ghofraan A. Ata'Allah, Noor Azmi Mat Adenan, Mukhri Hamdan

Department of Obstetrics and Gynaecology, Faculty of Medicine Universiti Malaya, 59100 Kuala Lumpur, Malaysia

Abstract

Objectives: To determine the proportion of immature (IM) oocytes or post-mature (PM) in stimulated cycles and to investigate the possibility of their utilization to prevent wastage.
Background: In our center, we normal perform conventional in vitro fertilization-in vitro maturation (cIVF-IVM) for IM oocytes while PM oocytes with normal appearance are subjected to cIVF or intracytoplasmic sperm injection (ICSI).
Subjects and methods: Apparently mature oocytes with good and healthy cumulus were subjected to cIVF or denuded for ICSI. All IM oocytes (GV & MI) were subjected to cIVF-IVM. IVM was performed in fertilization medium with cumulus co-culture.
Results and Discussion: The number patients were 54. The number oocytes retrieved were 501; 52 (10.4%) were GV, 46 (9.2%) were MI, 388 (77.4%) were MII and 15 (3%) were PM. For MI oocytes, 20 of 46 (43.5%) fertilized with visible PNs and 17 (85%) cleaved. Six (6) of 17 (35.3%) embryos from MI oocytes (with average of 6-cells, grade A) were transferred back to 5 patients that also received embryos from MII oocytes. Two patients became pregnant. Both patients received 1 embryo each from MI and MII oocytes. One patient became pregnant with triplets but miscarried at second trimester. While the other patient is currently pregnant with a singleton. Of the remaining embryos from MI oocytes 3 of 11 (27.3%) embryos developed to 2 compacted morulae and 1 blastocyst and were frozen. Of the 52 GV oocytes subjected to cIVF-IVM none fertilized. Since embryos from both MI and MII oocytes were transferred, it is not certain MI oocytes contributed to pregnancies but the possibility exist.
Conclusion: Immature oocytes can be rescued by cIVF-IVM to generate quality embryos that can be used by the patients. Discarding immature oocytes is loss to the patients and may be ethically questionable. It is shown that about 19.6% of the oocytes are IM and 3% are PM in stimulated cycles.

Disclaimer: The authors have no conflicts of interest.
J Reprod Biotechnol Fertil 13:49-51
Correspondence: Siti Khadijah Idris; **Email**: khadijahid@ummc.edu.my
Compliance acknowledgement: This article was edited by the Australian Editorial Services (www.nativeenglisheditor.com)
Keywords: Embryo, fertilization immature, oocytes, rescue

Introduction

Approximately 15-30% (Ali et al., 1998; Huang et al., 1999; Ali, 2009; Mendelbaum et al., 2021; Shin et al., 2013; Rubino et al., 2013; Junca et al., 1995;) of the oocytes are anticipated to be immature (IM), oocytes, namely the metaphase I (MI), and germinal vesicle, (GV) oocytes. Likewise, a proportion of the oocytes are expected to be post-mature (PM). Most centers utilize mature oocytes while immature and post-mature oocytes, the latter with grossly abnormal features combined with a lack of resistance to the injection pipet, are normally discarded. The under-utilization of immature oocytes in IVF treatments is a loss to the patients.

In our center, we normally perform combined conventional in vitro fertilization and in vitro maturation (cIVF-IVM) for IM oocytes while PM

oocytes with normal appearance are subjected to cIVF or intracytoplasmic sperm injection (ICSI) as previously described (Ali, 2009).

The report of Ali (Ali, 2009) have noted these immature oocytes could be matured in vitro by simple in vitro maturation (IVM) technique in which metaphase I oocytes (12 of 12) in ultra-microdrop (UMD) culture (Ali, 2004) of about 1.5–2 mL (4) promoted maturation within 24 hours in standard embryo culture medium (Cook IVF Cleavage Medium, Limerick, Ireland).

The same study also noted that oocytes at the germinal vesicle stage that grew under comparable circumstances showed a slight decrease in maturation at 24 hours (39.4%, 13 of 33) but improved after 48 hours of IVM (48.5%, 17 of 30).

When autologous granulosa cells (GC) and/or cumulus cells (CC) coculture was used in 2.5–3.0 mL of UMD culture (Ali, 2004), under identical circumstances as IVM, a greater percentage of GV oocytes reached maturity at 24 hours (54.5%, 6 of 11) and 48 hours (81.8%, 9 of 11).

It is felt that the development of GV oocytes may be aided by paracrine substances that are released into the UMD coculture environment by the egg, GC, and CC concentrated inside the ultra-volume of the culture system. The findings of Ali (Ali, 2009) in UMD coculture were comparable to those obtained with specialized IVM medium containing follicular fluid, FSH, and hCG supplementation (Zhu et al., 2008).

However work in this area of rescue IVM has not been pursued because of the concern of asynchronous cytoplasmic and nuclear maturity (Combelles et al., 2002) that affects treatment outcome following IVM. The actual definition of oocyte maturity is synchronous nuclear and cytoplasmic maturation, which guarantees oocyte fertilization and developmental potential (Damiani et al. 1996; Fulka et al., 1998; Barnes et al, 2000).

The objectives of the present study was to determine the proportion of immature (IM) oocytes or post-mature (PM) in stimulated cycles and to investigate the possibility of their utilization to prevent wastage.

Subjects and methods

Apparently mature oocytes with good and healthy cumulus were subjected to cIVF or denuded for ICSI. All IM oocytes (GV & MI) were subjected to cIVF-IVM. IVM was performed in fertilization medium with cumulus co-culture as previously described (Ali, 2009). Grossly PM oocytes were rarely utilized (cIVF or ICSI) unless necessary especially if the patients have no other oocytes.

Results and Discussion

The number patients were 54. The number oocytes retrieved were 501. Of these, 52 (10.4%) were GV, 46 (9.2%) were MI, 388 (77.4%) were MII and 15 (3%) were PM. For MI oocytes, 20 of 46 (43.5%) fertilized with visible PNs and 17 (85%) cleaved. Six (6) of 17 (35.3%) embryos from MI oocytes (with average of 6-cells, grade A) were transferred back to 5 patients that also received embryos from MII oocytes.

Two patients became pregnant. Both patients received 1 embryo each from MI and MII oocytes. One patient became pregnant with triplets but miscarried at second trimester. While the other patient is currently pregnant with a singleton. Of the remaining embryos from MI oocytes 3 of 11 (27.3%) embryos developed to 2 compacted morulae and 1 blastocyst and were frozen.

Of the 52 GV oocytes subjected to cIVF-IVM none fertilized. As for PM oocytes, 7 of 15 (46.7%) fertilized by ICSI and all of them (100%) cleaved but were mostly not of good quality and were not selected for transfer as the patients had good quality sibling embryos derived from healthy mature sibling oocytes. Of the 7 embryos derived from PM oocytes, 2 (28.6%) become morulae but did not progress to the blastocyst stage and were not of good quality. GV oocytes could not be efficiently matured because none of the GV oocytes matured in vitro fertilized.

Conclusion

It is shown that about 19.6% of the oocytes are IM, 9.6% are MI, 10.4% were GV stage

oocytes and 3% are PM oocytes in stimulated cycles. MI oocytes can be rescued by cIVF-IVM to generate quality embryos that can be used by the patients, that could lead to pregnancies and may assist increase treatment success.GV oocytes could be matured but do not develop well. More work is required to utilize GV oocytes to prevent loss. Discarding immature oocytes is loss to the patients and may be ethically questionable.

References

Ali J, H.N. Joshi, M. Al-Badr, M.A.M. Shahata, A. Abdulkader, S. Al-Natsha, M. Flamerzi and A. Hamsho (1998) Subzonal insemination of immature oocytes generated during intracytoplasmic injection cycles. Med. Sci. Res. (UK) 26:389-391.

Ali J. Continuous ultra micro-drop (cUMD) culture yields higher pregnancy and implantation rates than either larger-drop culture or fresh-medium replacement. Clin Embryol 2004;7:1–23.

Ali J. In Vitro Maturation in Standard Embryo Culture Medium of Immature Oocytes Recovered During Stimulated Cycles. Middle East Fertility Society Meeting, Cairo, November 4 – 7, 2009.

Barnes FL, Sirard MA. Oocyte maturation. Semin Reprod Med. 2000;18(2):123–31. doi: 10.1055/s-2000-12551.

Combelles CM, Cekleniak NA, Racowsky C, Albertini DF. Assessment of nuclear and cytoplasmic maturation in in-vitro matured human oocytes. Hum Reprod. 2002 Apr;17(4):1006-16. doi: 10.1093/humrep/17.4.1006.

Damiani P, et al. Evaluation of developmental competence, nuclear and ooplasmic maturation of calf oocytes. Mol Reprod Dev. 1996;45(4):521–34.

Fulka J, Jr, First NL, Moor RM. Nuclear and cytoplasmic determinants involved in the regulation of mammalian oocyte maturation. Mol Hum Reprod. 1998;4(1):41–9. doi: 10.1093/molehr/4.1.41.

Huang FJ, Chang SY, Tsai MY, Lin YC, Kung FT, Wu JF, Lu YJ. Relationship of the human cumulus-free oocyte maturational profile with in vitro outcome parameters after intracytoplasmic sperm injection. Journal of assisted reproduction and genetics. 1999;16(9):483–487. doi: 10.1023/A:1020551000150.

Junca AM, Mandelbaum J, Belaïsch-Allart J, Salat-Baroux J, Plachot M, Antoine JM et al. Oocyte maturity and quality: value of intracytoplasmic sperm injection. Fertility of microinjected oocytes after in vitro maturation. Contraception, fertilite, sexualite (1992). 1995;23(7-8):463-465.

Mandelbaum RS, Awadalla MS, Smith MB, Violette CJ, Klooster BL, Danis RB, McGinnis LK, Ho JR, Bendikson KA, Paulson RJ, Ahmady A. Developmental potential of immature human oocytes aspirated after controlled ovarian stimulation. J Assist Reprod Genet. 2021 Sep;38(9):2291-2299. doi: 10.1007/s10815-021-02253-7.

Rubino P, Viganò P, Luddi A, Piomboni P. The ICSI procedure from past to future: a systematic review of the more controversial aspects. Human reproduction update. 2016;22(2):194–227. [PubMed] [Google Scholar]

Shin SB, Cho JW, Lee SH, Yang KM, Lim CK, Lee HS. Fertilization and pregnancy potential of immature oocytes from stimulated intracytoplasmic sperm injection cycles. Clinical and experimental reproductive medicine. 2013;40(1):7–11. doi: 10.5653/cerm.2013.40.1.7.

Zhu XM, Zhu YM, Xu CM, Qian YL, Jin F, Huang HF. Autologous mature follicular fluid: its role in in vitro maturation of human cumulus-removed oocytes. Fertil Steril 2008;90:1094–102.