Zona free or Zona damaged human oocytes: experiences and indications for their rescue

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Summary

Zona Pellucida (ZP) has both mechanical and active functions. Many aspects related to its participation in the fertilization process are still controversial and ZP anomalies remain a potential cause of infertility. The integrity of the female gamete is important to ensure its functions and competence. By chance, it is possible to find zona free (ZFO) or zona damaged (ZDO) oocytes caused by lack of synthesis or damages during manipulation for in vitro fertilization. This problem becomes relevant when few oocytes are retrieved or in particular clinical conditions (early menopause, severe endometriosis, oncology). Experiences and tricks to rescue these oocytes are presented in this work.

KEY WORDS: oocyte, zona damaged, zona free.

Introduction

The zona pellucida (ZP) is the outer part of the oocyte. It is an extracellular matrix constituted by different families of acidic glycoproteins. It plays both mechanical and active functions. The lack of ZP could be due to the absence of synthesis, to its fragility, to unsafe retrieval procedures or to damage during cumulus corona cells removal procedures.

The ability of ZFO to reach the blastocyst stage in vitro, was described in 1999 (1, 2). The first pregnancy with ZFO was reported in 2001 (3). Shortly later, the possibility to cryopreserve embryos coming from ZFO was described and confirmed by a pregnancy obtained after warming of a blastocyst vitrified after the complete escape from the ZP during development (4).

The rescue of ZFO or ZDO worth when the number of oocytes retrieved is very small or in patients in which ovarian stimulation is repeatable (early menopausal risk, oncologic, i.e.).

In this work, some experiences and tricks to rescue these oocytes are described.

Methods and results

Five cases (four patients) underwent ovarian stimulation for risk of early menopause or severe endometriosis, are described:
1) total ZP lack, PolScope evaluation, insemination, culture;
2) breakage or thin ZP, insemination, culture;
3) hemizona, PolScope, insemination, culture;
4) ZP breakage, vitrification, warming, insemination, culture;
5) oocyte totally escaped from ZP, PolScope evaluation, reconstruction, vitrification.

First case

34 years old. Only one oocyte was retrieved that was found ZFO during denuding procedure. A lack of synthesis was supposed because no ZP
debris was found. The first polar body (1°PB) was absent. For this reason, PolScope (Cri., Woburn, Ma) (Figure 1 a, b) was utilized to verify the nuclear maturity. After the visualization of meiotic spindle, Intracytoplasmic Sperm Injection (ICSI) was performed with great caution applying a very light suction with the holding pipette and entering very slowly with the injection pipette trough the oolemma. Fertilization check confirmed the presence of 2 pronuclei and one PB, considered the second one. The zygote was left in culture without further manipulations. Two pseudo-blastocysts were observed at day 6. They were not transferred into the uterus.

Second case (same patient)
Two oocytes were retrieved. Considering the first experience, oocytes were carefully observed and partially denuded (capillary >170 µm diameter). One oocyte showed a complete but very thin ZP. It degenerated after ICSI. The other one showed the lack of only a small part of the zona (Figure 2). It fertilized and a four cells embryo was transferred into the uterus at day 2. No pregnancy was achieved.

Third case
44 years old. Three oocytes were retrieved, one of them presented an hemizona. The two normal oocytes were inseminated. The hemizona oocyte was evaluated by PolScope and then microinjected with great caution. Hemizona was kept far during ICSI but maintained very close to the oocyte during culture (Figure 3 a-d). The two normal oocyte fertilized, the ZDO did not fertilized. Two embryos were transferred but no pregnancy was obtained.

Fourth case
33 years old. The patient had seven mature oocytes previously vitrified after an ovarian stimulation. In the first warming cycle four oocytes were warmed and survived. One was a ZDO showing a ZP rupture already observed before cryopreservation. The latter was injected exploiting the ZP cut to insert the injecting pipette to reduce the risk of damage it. The ZDO and another one fertilized normally, the third was abnormal and the fourth one failed to fertilize. Two embryos of five and six cells respectively were transferred on day three. No pregnancy was obtained (Figure 4 a-e).

Fifth case
35 years old. The patient underwent ovarian stimulation for oocytes preservation. Two oocytes were retrieved. One oocyte was rebuilt with the help of the micromanipulator after be

Figure 1 a, b - a) Zona Free Oocytes without 1° PB. b) Meiotic spindle visualization of the Zona Free Oocyte. The oocyte with ZP was positioned next to ZFO to improve the visualization of the inner structure of the latter (Poloscope 20X).

Figure 2 - The oocyte shows the lack of a part of the ZP. Some corona cells in the lower part were left in place to maintain a container function.
released completely from the ZP during the de-nuding procedure. Two holding pipettes were utilized: one to hold the ZP and the other to push the oocyte inside it. The flaps of the broken ZP were put as near as possible trying to reduce the opening. The oocyte was vitrified and not yet warmed (Figure 5 a-e).

Discussion

The female gamete integrity is important for its functions and competences. ZP has several functions but the containing one is very important to ensure the correct connection and contact among cells until the blastocyst hatching. Even irregular or abnormal zona pellucida can affect negatively the outcomes (5). Some attempts can be done to restore its integrity when it is lost for different reasons. The utilization of donated human ZP presents immunological, ethical and legal problems, the assembly of a synthetic ZP will be conceivable in the future. Actually, in cases of complete lack of ZP, it is advisable to leave some corona cells around the oocyte that play the container role. A beautiful time lapse monitoring that shows the develop of ZFO until blastocyst is available online (6). The potential of oocytes with ZP damaged was evaluated in another work. They removed completely the damaged ZP and ZFO were fertilized and successfully cultured until blastocyst stage (7). A rare case of complete ZFO in all the retrieved oocytes is described in a recent work. A pregnancy was obtained and the Authors recommend caution on the number of embryo selected to reduce multiple pregnancies (8).

What we try to do in this work, was to reconstitute the damaged oocytes when possible because we believe in the importance of the female gamete integrity.

It is difficult to diagnose a ZFO until the cumulus corona complex is present. It is possible to foresee it only if the patient had the same problem in previous cycle or if some of the oocytes show this problem during denudation. Precau-
tions consist in:
- to manipulate the oocyte with great caution,
- to make only a partial decoronization (low concentrated hyaluronidase (20-40 UI/ml);
- to utilize commercial capillary with a known diameter not less than 170 µm;
- to move them as less as possible.

1°PB is quite always lost in ZFO. For this reason it is strongly advised to verify the presence of meiotic spindle by PolScope before insemination to check the nuclear maturity. The trick of putting a normal oocyte with ZP near ZFO, is advised to make possible a good visualization of meiotic spindle of the latter. In this way, the ZP reflectivity, capture enough light to lead the clear visualization of the inner structure of ZFO. Sometimes exists the possibility to reconstitute the oocyte utilizing the broken ZP in cases of breakage during manipulation. If the oocyte is completely escaped from ZP, it is possible to re-build it. In this way no damages are caused to the fragile oolemma.

Furthermore, it is mandatory to apply a very low suction with the holding pipette to not damage

Figure 4 a-e - The oocyte shows a ZP rupture that was exploiting to insert the injecting pipette during ICSI. The last figure shows the embryo developed after normal fertilization. The cut is still evaluable.
the oolemma and take care to avoid the introduction of residual corona cells into the cytoplasm during ICSI.

Conventional in vitro insemination is not recommended because the polyspermy block is altered by the absence of ZP.

After insemination, oocytes have to be manipulated as less as possible using pipettes with big diameter (Pasteur pipette or capillary 300µ diameter).

ZFO oocytes or the developed embryos, can be cryopreserved using conventional techniques as previously reported even if, they are probably more susceptible to contaminations during cryopreservation and storage (4).

The Literature and our experiences confirm that it is possible to recover ZF or ZP damaged human oocytes. They fertilize and cleave normally and they are eligible for cryopreservation when needed. To maintain the correct cell-to-cell contacts due to the presence of ZP and perivitelline space, it is important: to reconstitute the oocyte integrity when possible, or to leave some corona cells layers around it. It is mandatory to manip-

Figure 5 a-e - This oocyte was completely escaped from ZP. The reconstruction was done utilizing two holding pipettes: one to hold the zona and the other to push the oocyte inside it. The flaps of the broken zona were put as near as possible trying to reduce the opening during culture.
ulate these oocytes with great caution, to move them as little as possible and to check the presence of the meiotic spindle before insemination for the evaluation of nuclear maturity. Furthermore, it is recommended to reach the blastocyst stage before transfer in case of complete ZP absence. Few cases are reported in Literature and more experiences are needed to verify the outcomes. Cases in which the rescue of these oocytes is needed will be more frequent in IVF laboratories considering the increasing mean age of patients and the increasing requests of fertility preservation.

References


