Case Report: Pregnancy outcome following ICSI of oocytes with abnormal cytoplasm and zona pellucida

Gedalia Paz1,3, Ami Amit2 and Haim Yavetz1

1Institute for the Study of Fertility and 2IVF Unit, Lis Maternity Hospital, Tel Aviv Sourasky Medical Center, and Sackler Faculty of Medicine, Tel Aviv University, Israel
3To whom correspondence should be addressed at: Institute for the Study of Fertility, Lis Maternity Hospital, Tel Aviv Sourasky Medical Center, 6 Weizman Street, Tel Aviv 64239, Israel. E-mail: paz@tasmc.health.gov.il

A couple with unexplained infertility was referred for routine IVF and ICSI treatment. Ovulation was induced by the GnRH analogue protocol combined with HMG and HCG. Preparation of denuded oocytes revealed a major disorder of the zona pellucida and abnormal oocytes. During preparation of ova for ICSI, 15 retrieved oocytes were denuded, 14 of which underwent ICSI treatment. Four of the oocytes collapsed and the remaining 10 appeared to have irregular, fragile zona pellucida. Nevertheless, following ICSI, seven low-quality embryos developed, three of which were transferred into the uterus. Two implantations were achieved, but only one embryo resulted in an uneventful pregnancy and delivery by Caesarean section of a normal female neonate with an Apgar score of 10. It is hypothesized that infertility was due to the abnormal oocyte structure and abnormal zona pellucida, which prevented natural conception. This condition was successfully resolved by the ICSI procedure.

Key words: ICSI/oocyte/sperm/unexplained infertility/zona pellucida

Introduction

The term 'unexplained infertility' in a couple with no obvious male or female infertility factors is used to define idiopathic infertility of more than 2 years' duration. A couple diagnosed with unexplained infertility is referred for ovulation induction and intrauterine insemination (IUI) of washed sperm (Speroff et al., 1999; Pandian et al., 2001). If this treatment fails, routine IVF, which has a good chance of achieving pregnancy, is offered to the couple. Nevertheless, even in this situation, defective gametes may be found using more advanced methods, such as zona pellucida binding assay or zona pellucida acrosome reaction (AR), as suggested by Liu et al. (1997).

This case report describes a couple classified as having unexplained infertility, and referred for treatment to an IVF unit.

Case report

A couple presenting with primary infertility after 6 months of unprotected intercourse was referred to the outpatient clinic of the Institute for the Study of Fertility. Analysis of ejaculated semen of the 31-year-old male revealed semen of excellent quality (volume 3.5 ml; concentration $10^6$/ml; motility 65%; vitality 75%; and 18% of normal forms according to strict criteria).

The non-smoking, 29-year-old, healthy female with regular cycles (height 1.60 m and weight 60 kg) was evaluated using routine parameters including complete hormonal profile (prolactin, FT4, FSH, LH and testosterone) and full cycle evaluation. Tubal patency was confirmed by hysterosalpingography. The results of all tests were within the normal range, confirming the existence of spontaneous ovulations. The female was superovulated for four cycles with a treatment protocol of clomiphene citrate and HCG, and for three cycles with HMG and HCG. Monitoring was performed by ultrasonography, measurements of blood estradiol and progesterone. Intrauterine inseminations were scheduled accordingly, using the husband’s washed sperm suspension, but pregnancy was not achieved.

Initially, the infertile couple in this study was defined as having unexplained infertility since both partners were within the normal range of fertility, as confirmed by common, routine tests, and since the female failed to conceive after repeated induced superovulation and IUI. This definition was confirmed in the past, and has been summarized elsewhere (Speroff et al., 1999; Pandian et al., 2001). In the event of failed IUI treatments, IVF-ICSI has previously been offered to the couples (Liu et al., 1997); the same protocol was performed in the present report.

The couple was classified as having unexplained infertility and referred to the IVF unit for further treatment. Ovulation induction was performed using routine protocol of GnRH analogue (900 μg of buserelin acetate nasonal spray; Superfact; Hoechst AG, Frankfurt, Germany) combined with two ampoules of HMG (Pergonal; Teva Pharmaceutical Industries...
Ultrasound-guided oocyte retrieval was performed 36 h after HCG administration (Chorigon; Teva Pharmaceutical Industries Ltd) and yielded 15 cumuli, including oocytes of normal appearance. The oocytes were scheduled for insemination by routine IVF, as the sperm cells and the cumulus complex appeared to be of good quality. Nevertheless, at the couple’s request to perform five of the fertilizations by ICSI, the oocytes were prepared for injection. During preparation of the ova for ICSI, the cumulus was removed by repeated aspiration into glass pipettes in modified human tubal fluid with 0.025% hyaluronidase type 7 (Sigma Chemical Co., St Louis, MO, USA). The appearance of the oocytes was abnormal, with irregular thickness of the zona pellucida, and granular ooplasm (Figure 1A and B). The embryologist decided to process all the oocytes using the ICSI method. Denuded oocytes were thoroughly rinsed and injected with an isolated sperm cell. The embryologist observed that four of the 14 oocytes did not survive the procedure and collapsed due to extreme zona pellucida fragility. Of the 10 denuded oocytes treated, seven were fertilized. The appearance of the fertilized 2PN embryos (Figure 1C) was abnormal, like the oocytes, compared with a normal 2PN embryo taken from another patient (Figure 1D). The other three oocytes were damaged, and collapsed during the ICSI process. Seventy-two hours after ICSI, all seven embryos were incubated for 5 days, but only one blastocyst developed, which was normal in appearance (Figure 1F). Upon thorough inspection of the oocytes and the embryos it was noted that the zona pellucida was extremely irregular in shape. The density of zona pellucida was variable, with some areas of thin and loose membrane that may have contributed to the zona pellucida fragility, as well as having projections of the granular ooplasm, which appeared with profound dense spots (Figure 1A), considerably different from a normal one, all of which may hint at plasma membrane dysfunction.

Three embryos were transferred into the uterus. A pregnancy of two embryos was observed 4 weeks after transfer (based on gestational sacs). However, only one embryo survived and developed normally, leading to delivery by Caesarean section of a healthy baby girl, at term.

Discussion

Unexplained infertility is a syndrome the definition of which is rapidly changing with the advancements in the assisted reproductive technology. Recently, Levran et al. (2002) reported eight cases of unexplained infertility treated with inseminations, but without success. IVF treatment of the couples revealed that oocyte maturation was arrested at different stages of meiosis and failed to progress independently. In these cases, the use of oocyte donation was suggested, and achieved excellent results.

In our study, we initially found no proof of oocyte defects, due to the normal appearance of the retrieved expanded oocyte–cumulus complexes. A defective structure of the zona
pellucida was discovered only during the ICSI procedure when the oocytes were denuded.

Veeck (1988) described the normal ‘ideal’ configuration of a fertile oocyte. A metaphase II oocyte should have a clear, moderately granular cytoplasm, a small perivitelline space and a clear, colourless zona pellucida. No attention has been given to the appearance of the zona pellucida.

Stripping the cumulus cells that surround the zona pellucida is important during ICSI procedure. Only then is it possible to assess the quality of the oocyte. In our case, the aspirated oocyte–cumulus complexes looked normal after retrieval, but appeared abnormal and collapsed following treatment. Thus, the cumulus cells kept the abnormal oocytes with the irregular zona pellucida in the oval normal shape due to its firm organization with the extracellular matrix. This structure collapsed after stripping, due to the weakness of the wall formed by the irregular zona pellucida.

If the fertilized oocyte survives the stripping of the cumulus cells and the injection of the sperm, it divides into the morula stage and becomes normal in appearance, surrounded by the abnormal zona pellucida (Figure 1E). Thus, during the first stages of embryogenesis the blastomeres are organized in a way that caused the formation of an oval configuration, despite the presence of a weak wall (irregular zona pellucida). From this stage, normal development of the blastocyst (Figure 1F) and implantation of the embryo took place, as described.

There are controversial reports regarding the relationship between oocyte morphology, fertilization rates, quality of embryos and pregnancy rates.

It has been reported that oocyte morphology is not related to fertilization rates or embryo quality (De Sutter et al., 1996; Balaban et al., 1998), or on the development of the embryo, although high rates of aneuploidy have been found in these embryos (Kahraman et al., 2000). On the other hand, others have shown that oocytes with poor morphology, which may also be a result of the immaturity of the cytoplasm, consequently resulted in the formation of poor-quality embryos, which led to low pregnancy rates (Sehral et al., 1997; Xia, 1997; Loutradis et al., 1999). No special attention has been devoted to the zona pellucida of oocytes.

It is well established that binding of sperm cells to the zona pellucida glycoprotein-3 (ZP3) is the first stage in the fertilization process, and is followed by secondary binding to ZP2 (Wassarman, 1999). Thereafter, AR occurs, leading to penetration of the zona pellucida by the sperm cells and fusion of the sperm and the oocyte membranes. Liu et al. (1997) described cases in which, despite normal sperm quality and binding of sperm to the zona pellucida, no fertilization occurred. Such patients usually have a long duration of unexplained infertility, which can be treated successfully with ICSI.

We did not perform the zona pellucida binding test in the present case, since the woman conceived on the first ICSI cycle. This test will be suggested to the couple on their next visit to the IVF centre.

The normal integrity of the zona pellucida is a prerequisite for appropriate oocyte–sperm interaction. Study of the mouse model of zona pellucida matrix suggests that ZP2 and ZP3 are cross-linked by homodimers of ZP1 (Greve and Wassarman, 1985).

A model of mutant mice has shown that all three glycoproteins (ZP1, ZP2 and ZP3) are essential for normal fertility. The absence of one glycoprotein will cause decreased fertility. The most important glycoprotein is ZP3, of lesser importance is ZP2, and ZP1 is the least important for successful fertilization. The absence of zona pellucida glycoproteins also affects the quality of the mouse oocytes, causing fertilization failure (Rankin et al., 2001). The variable density and thickness of the zona pellucida of denuded oocytes described in our study may hint at the existence of an irregular mixture of the different zona pellucida glycoproteins. This is only speculation, and further studies are needed to confirm this hypothesis.

In the present case the sperm quality of the husband was found to be excellent for all routine parameters. For this reason, no functional tests such as induced acrosome reaction or zona pellucida binding assay with donor oocytes were carried out. In addition, the woman conceived on the first IVF-ICSI, cycle so there was no possibility to perform such tests with the abnormal oocytes of the woman.

The main cause of the infertility of the couple described in the present study was discovered during the ICSI procedure. The oocytes were found to possess a defective zona pellucida, in addition to fragility of the entire oocyte.

In conclusion, the infertility of the couple in our study did not have ‘unexplained infertility syndrome’, but rather infertility due to abnormalities in the cytoplasm of the oocyte and in the zona pellucida integrity. It is suggested that oocytes affected with this anomaly should not be discarded, but rather should be treated using the ICSI procedure. The blastocysts that subsequently develop, although delayed in their cleavage, can undergo normal implantation once they reach the uterus.

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References


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