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Evidence of parthenogenetic origin of ovarian teratoma: Case report

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This case report represents one of the few documented cases of parthenote embryo retrieval from an IVF patient with a history of ovarian teratomas. A 29-year-old woman presented at our centre with a history of primary infertility for 6 years due to male factor. She had undergone left oophorectomy 4 years before due to an ovarian teratoma. An ultrasound scan performed during basal evaluation revealed two complex images in the right ovary suggesting teratomas, measuring 2.5 × 2.4 and 1.7 × 1.3 cm. A significant extent of sonographically normal ovarian parenchyma was present, and the patient underwent the long leuprolide acetate protocol of ovarian stimulation with recombinant FSH for an IVF-ICSI cycle. She had 13 metaphase II (MII), four metaphase I (MI), two germinal vesicle (GV) oocytes and one 4-cell embryo retrieved. Eight out of nine injected oocytes were fertilized normally while one was unfertilized. Embryo transfer was carried out 72 h after retrieval. The 4-cell (parthenote) embryo recovered at oocyte retrieval continued to cleave in culture, developing into a 7-cell embryo by the next day. The embryo was morphologically normal, presenting an evident nucleus in each blastomere. Fluorescent *in situ* hybridization (FISH) returned two signals for the X chromosome in each blastomere that was analysed. Of the eight normally fertilized embryos, three were transferred, resulting in a normal singleton pregnancy and the birth of a healthy baby.

Key words: embryo/FISH/IVF-ICSI/ovarian teratoma/parthenogenetic oocyte activation

Introduction

Teratomas are tumours with more than one cell type which originate from more than one germ layer. These cells may differentiate into any tissue of the body including hair, teeth, fat, skin, muscle and others. These often bizarre tumours are usually located at the midline and paraxial regions of the body. One of the most common locations is the ovary, although they may also occur in the testes (Crum, 1999).

Ovarian teratomas are habitually named dermoid cysts. Mature cystic teratomas account for 10–20% of all ovarian neoplasms, and most of them are benign. They are the most common ovarian neoplasm in young patients and affect both ovaries in 10–15% of cases (Crum, 1999). Although usually benign, malignant transformation occurs in ~1% of cases (Te Linde and Mathingly, 1977).

The initial biological event which leads to such a heterogeneous and bizarre tumour is not yet understood. However, it is believed that ovarian germ cell tumours (including teratomas) may develop directly from oocytes. Also, a high frequency of ovarian teratomas has been described in some inherited mutant mice (strains LT/Sv and *Mos*-null). In these strains, ovarian teratomas are derived from oocytes that undergo maturation and spontaneous parthenogenetic activation followed by embryonic development within the ovarian follicles (Stevens and Varnum, 1974; Hirao and Eppig, 1997).

The most accepted theory to explain the existence of ovarian teratomas is the parthenogenetic activation of oocytes. The fact that the 46,XX karyotype is found in almost all mature teratomas strengthens this theory. Even though embryonic development without the male gamete, named parthenogenesis, can be found in some lower organisms, this sort of reproduction seems to be completely unsuccesful in mammals. Nevertheless, this does not mean that embryonic tissues cannot be generated from parthenogenetically activated oocytes in humans. Although all germ layer derivatives are encountered in this kind of tumour, it is known that mammalian parthenotes fail to proceed to a successful pregnancy.

The finding of a spontaneously activated developing embryo, retrieved from an intact follicle, in patients with ovarian teratomas so far has not been described frequently

(Padilla et al., 1987). Our aim is to report the observation of a spontaneously activated developing embryo in a patient with diploid ovarian teratomas, suggesting a possible link between spontaneous parthenogenetic activation of oocytes within the ovary and teratoma development. Thus, we hope to contribute to a better understanding of the mechanisms involved in the pathogenesis of these frequently observed tumours in young women.

Case report

A 29-year-old woman presented at our centre with a history of primary infertility for 6 years due to male factor (severe oligospermia). A hysterosalpingogram performed 6 months before had revealed bilateral tubal patency. She had undergone left oophorectomy 4 years before due to an ovarian teratoma. On that occasion, pathological examination revealed a cystic mature teratoma. Cytogenetic analysis of the ovarian teratoma was performed and the genotype was 46,XX.

An ultrasound scan performed during basal evaluation at our centre revealed two complex images in the right ovary which suggested the diagnosis of teratomas, measuring 2.5 × 2.4 and 1.7 × 1.3 cm. Despite such findings, a significant extent of sonographically normal ovarian parenchyma was present. After a discussion of the case with the patient, a decision was made to perform an immediate IVF-ICSI cycle. She underwent a long leuprolide acetate protocol, starting on day 21 of the previous cycle with a dosage of 0.5 mg/s.c. twice a day for 3 days followed by 0.5 mg s.c. per day for 11 days. After assessment of pituitary downregulation [estradiol (E_2) <30 pg/ml], ovarian stimulation was initiated with recombinant FSH (Gonal F, Serono, São Paulo) in a dosage of 225 IU s.c. per day for 7 days. Subsequent recombinant FSH dosages were titrated according to daily monitoring of follicular development by transvaginal ultrasound and serum E_2 measurements.

The oocyte retrieval occurred without complications. The patient had 13 metaphase II (MII), four metaphase I (MI), two germinal vesicle (GV) oocytes and one '4-cell embryo' (parthenote) (Figure 1a) retrieved from an intact follicle during the oocyte retrieval. Eight out of nine injected oocytes were fertilized normally and presented two distinct polar bodies, while one was unfertilized. Three out of four MI oocytes became MII. The parthenote continued to cleave in culture, developing into a good quality 7-cell embryo ($<10\%$ fragmentation) by the next day (Figure 1b). It was morphologically normal, presenting an evident nucleus in each blastomere.

Blastomere biopsy was performed in the 7-cell parthenote utilizing zona drilling by laser (Fertilase, MTG, Medical Technology, Altdorf, Germany). The embryo was fixed by the holding pipette, and three pulses of 5–8 ms (1.48 nm) were applied after corrected positioning of the zone pellucida using the target generator. After zona drilling, blastomeres were aspirated one by one, removed from the embryo and released into the medium. Each intact blastomere was submitted to fluorescent *in situ* hybridization (FISH) for sexing, with probes for chromosomes X (Vysis, CEP X alpha satellite, Xp 11.1–q11.1) and Y (Vysis, CEP Y alpha satellite, Yp11.1–q11.1). FISH analysis performed on six out of seven blastomeres returned two signals for the X chromosome (one blastomere degenerated due to biopsy procedure).

Figure 1. (**A**) A human embryo at the 4-cell stage which was retrieved from an intact follicle on the day of oocyte pick-up in a patient with an ovarian teratoma. (**B**) The same embryo (at the 7-cell stage) the day after the oocyte pick-up.

Embryo transfer was carried out 72 h after oocyte retrieval. Three good quality embryos ($<10\%$ fragmentation, eight regular blastomeres) were transferred, resulting in a normal singleton pregnancy and a healthy baby.

Discussion

The finding of a 4-cell stage embryo (parthenote) retrieved from an intact ovarian follicle in a woman with antecedent of diploid ovarian teratoma (46,XX) is unlikely to be coincidental. Although the presence of an ovarian cystic teratoma in patients undergoing IVF-embryo transfer (ET) is not a common finding, Caspi et al. (1998) reported a series of cases in which IVF-ET cycles and induction of ovulation were performed in patients with ovarian cystic teratomas. They concluded that such procedures could be performed safely and successfully in these patients. Thus, it is important to highlight that IVF-ET is a feasible procedure in patients with ovarian teratomas.

The overall frequency of spontaneously parthenogenetic activated oocytes retrieved from intact ovarian follicles in patients undergoing IVF-ET is unknown. In a close retrospective analysis of our own data of 6000 IVF-ICSI cycles from January 1995 to December 2003, such abnormal oocytes/embryos (parthenotes) have never been retrieved from an intact ovarian follicle (R Abdelmassih, V Abdelmassih, FG Oliveira, ZP Nagy, S Abdelmassih, unpublished data). It has not yet been established whether there is a relationship between such abnormal oocytes/embryos and the co-existence of an ovarian teratoma. Besides, it is not known if the drugs used in ovarian stimulation, such as recombinant gonadotrophins and GnRH agonists, may induce parthenogenesis in assisted reproductive technology (ART) patients. However, a possible association between a spontaneous parthenogenetically activated oocyte retrieved as a developing embryo and ovarian cystic teratomas has been described elsewhere. There was one case similar to ours in which a 2-cell parthenogenetic embryo was aspirated at oocyte retrieval for IVF in a woman with a history of bilateral cystic teratomas (Padilla et al., 1987).

The anatomopathological features of ovarian mature teratomas are well known. Different tissues such as hair, fat, thyroid and bones are often found and identified even macroscopically. However, the biological event which leads to such a heterogeneous and bizarre tumour is far from being understood. As it is, several theories have already been suggested, but none of them has yet been proved. Among these theories, it is worth mentioning the germ cell origin (Linder and Power, 1970), neoplastic proliferation of sequestered totipotent cells, incomplete twinning and depression of totipotent genetic information in the nuclei of somatic cells (Scully, 1979; Gonzales-Crussi, 1982).

In our patient, a cytogenetic analysis of the previously resected teratoma on the left ovary had revealed a 46,XX genotype. Nowadays, this happens to be a frequent practice due to the important information that can be provided with regard to the malignant potential of these tumours and karyotype abnormalities. Early studies detected a homozygous genotype in these tumours, suggesting that they are composed of germ cells that have undergone meiosis I (Eppig et al., 1977; Patil et al., 1978).

This subject, however, has been a matter of controversy. A pre-meiotic or somatic origin has also been suggested, since heterozygous genotypes have been found in mature ovarian teratomas (Surti et al., 1990). It was then suggested that alternative mechanisms should be considered in addition to the above-mentioned germ cell meiosis theory. Aiming to provide further light into this intriguing question, a study from Vortmeyer et al. (1999) applied tissue microdissection followed by genetic analysis of teratomas. The DNA was extracted from the tissue samples from seven patients and then amplified by PCR. A panel of genetic markers for different chromosomes was used to evaluate heterozygosity and homozygosity. The study concluded that ovarian teratomas present genetic homozygosity in the procured tissues, which supports the original theory of a germ cell origin.

The FISH analysis of the parthenote retrieved from an intact follicle in our patient resulted in a XX signal on day 2 of culture (7-cell embryo). Although we did not perform a complete karyotype of this embryo, the XX signal revealed by FISH in six out of seven blastomeres could be associated with the 46,XX genotype of the MI oocytes of the patient. All in all, we must accept the fact that, similar to the origin of most ovarian cystic teratomas, the 4-cell embryo recovered from our patient could have been generated by spontaneous parthenogenetic activation of an MI oocyte within the ovarian follicle (Eppig et al., 1977, 1996).

Since parthenogenetic activation of MI oocytes takes place in the ovarian follicles, there are probably other factors which contribute to the development and growth of the parthenote. It was shown that these abnormal embryos may develop to form the bizarre masses which consist of a variety of differentiated cell and tissue types—a unique feature of the ovarian teratomas. The ability of the mice parthenotes to adhere to the ovarian tissue and to differentiate into tissues from three germ layers, possibly by the action of ovarian factors synthesized by the corpora lutea, has been shown by Noguchi (1984).

Whether the development of ovarian teratomas may be increasingly associated with ovarian stimulation in ART is not known. However, one can postulate that an abnormal microenvironment created in the ovarian follicles by the regimens of stimulation with gonadotrophins and GnRH analogues may be associated with paracrine and/or autocrine effects which might be adjunctive factors in the development of parthenotes.

We therefore believe that our data and the evidence from other authors strengthen the theory of the origin of ovarian teratomas from oocytes activated during the transition between MI and MII. This origin is probably identical to that of teratomas described in some 30% of ovulatory oocytes of LT/Sv mice and may suggest a similar underlying mechanism. Our data thus provide further evidence supporting a parthenogenetic origin of ovarian teratomas. Also, it is important to highlight that patients with small size ovarian teratomas with a large extent of tumour-free ovarian parenchyma may be safely and successfully submitted to IVF-ICSI.

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