J Assist Reprod Genet (2013) 30:761–764
DOI 10.1007/s10815-013-0002-5

ASSISTED REPRODUCTION TECHNOLOGIES

Partial molar pregnancy after intracytoplasmic sperm injection occurring as a result of diploid sperm usage

Philip Savage · Neil Sebire · Tom Dalton · Anna Carby · Michael J. Seckl · Rosemary A. Fisher

Received: 4 March 2013 / Accepted: 16 April 2013 / Published online: 3 May 2013

Abstract
Purpose Partial molar pregnancies are rare conceptions characterized by having 69 rather than 46 chromosomes, the additional chromosome complement usually occurring as a result of fertilization of the ovum by two sperm. Although assisted conception with intracytoplasmic sperm injection (ICSI) should prevent the development of a partial molar pregnancy, occasional cases have been described after assisted conception using ICSI. The objective of this study was to investigate the cause of partial molar pregnancy in a couple who had undertaken assisted conception with ICSI.
Methods Fluorescent microsatellite genotyping of DNA from the couple and tissue from their partial molar pregnancy was performed in order to confirm diagnosis and investigate the origin of the additional chromosome set.
Results Genotyping confirmed that the partial molar tissue was triploid with an additional chromosome complement from the father. Genotyping of additional loci proximal to the centromere demonstrated that the two paternal sets of chromosomes originated in a single sperm with a double complement of paternal DNA resulting from non-reduction at the second meiotic division.
Conclusions This study confirms that partial molar pregnancy may occur after assisted conception with ICSI and that this occurs as a result of fertilization with a diploid sperm.

Keywords Assisted conception · ICSI · Molar pregnancy · Trophoblast · Genetic analysis

Capsule Triploid, partial molar pregnancies that develop after assisted conception using intracytoplasmic sperm injection result from the use of a diploid sperm, rather than failure of the fertilization process.

P. Savage · N. Sebire · T. Dalton · M. J. Seckl · R. A. Fisher
Department of Medical Oncology, Imperial College Healthcare NHS Trust, Charing Cross Hospital, London, UK

A. Carby
Department of Reproductive Medicine, Imperial College Healthcare NHS Trust, Hammersmith Hospital, London, UK

R. A. Fisher
Institute of Reproductive & Developmental Biology, Imperial College London, Hammersmith Hospital, London, UK

P. Savage (✉)
Consultant in Medical Oncology, Imperial College NHS Trust, Charing Cross Hospital, London W6 8RF, UK
e-mail: philip.savage@imperial.nhs.uk

Introduction

Assisted conception using in vitro fertilization (IVF) was developed in the late 1970s [1] and is now widely used to overcome fallopian tube blockages and sperm defects of number, function and motility. In 2010 over 45,000 women received IVF treatment in the UK with IVF births comprising nearly 2% of the UK total [2]. In assisted conception with intra-cytoplasmic sperm injection (ICSI) a single sperm is introduced directly into the ovum in vitro and overcomes many potential issues with sperm motility. This technology has been in use for over 20 years and appears to have no significant adverse outcome issues [3].

Partial molar pregnancies occur in approximately 1 in every 800 conceptions [4] and are characterized by a specific chromosomal defect in which there are 69 chromosomes, 46 of paternal origin, which typically occurs as a result of two individual sperm fertilizing a normal haploid ovum [5–7]. Molar pregnancies carry an appreciable risk of malignant change with approximately 10% of patients with complete and 1% of partial moles going on to require chemotherapy.

In vitro fertilization using ICSI should theoretically provide a protective effect against partial molar pregnancies by ensuring that only a single sperm fertilizes the ovum. However, cases of partial molar pregnancies occurring after ICSI have been reported [8, 9]. In these and other similar cases the patient has to cope with the failure of the pregnancy, the

risk of malignant change and the concept that a procedure that involves the injection of a solitary sperm has lead to a condition that generally results from two sperm fertilizing the same ovum. We report a case of partial molar pregnancy occurring after ICSI that confirms the observation that partial molar pregnancy can occur after ICSI and that this may result from the inadvertent use of a diploid sperm.

Materials and methods

A previously fit 29 year old woman with no prior conceptions underwent IVF treatment with ICSI for male factor fertility issues and had two embryos transferred in an uncomplicated procedure. Ten days later she obtained a positive pregnancy test. The first 8 weeks of the conception were uneventful, with an early ultrasound indicative of twins. A few days later the patient experienced some PV bleeding and further investigations suggested an incomplete miscarriage and a uterine evacuation was performed. Histopathological examination revealed a partial molar pregnancy and in keeping with UK practice, the patient was registered with the UK Trophoblastic Tumour Screening and Treatment Centre based at Charing Cross Hospital in London for ongoing monitoring of human chorionic gonadotrophin (hCG) and histopathological confirmation. Post evacuation her hCG levels fell to normal within 4 weeks and no additional treatment was required.

Subsequent to this ICSI assisted pregnancy the patient has had a successful natural conception and a healthy baby with her new partner, whilst her previous partner has become a father via ICSI with his new partner.

Genetic analysis of the molar tissue

To examine the genetic origin of the molar tissue, fluorescent microsatellite genotyping was performed using DNA from the patient, her partner and the molar tissue. Parental DNA was extracted from blood samples using a Qiagen blood mini kit (Qiagen, Sussex, UK) while DNA was prepared from molar villi microdissected from formalin-fixed, paraffin–embedded sections as previously described [10]. Following PCR amplification of DNA from the parents, with a panel of 23 primers for short tandem repeats (STRs) on different chromosomes, PCR products were resolved by capillary electrophoresis using an ABI 3100 Genetic Analyser (Applied Biosystems, Warrington, UK) and genotypes determined using GeneMapper software (Applied Biosystems, Warrington, UK). DNA from the molar tissue was then amplified with informative microsatellite markers i.e. markers for which all alleles in the parents DNA were different and the paternal DNA sample was heterozygous. The genotype of the molar tissue was compared with that of the parents to identify the origin of chromosomes in the molar tissue.

In order to refine the origin of the parental contributions to the molar tissue, DNA from the patient and her partner were subsequently amplified with a second panel of STRs at loci proximal to the centromere of the 22 autosomes. DNA from the molar tissue was then amplified with primers for a further 11 loci for which the paternal DNA was heterozygous. The genotype of the molar tissue was determined as above.

Results

Histopathological review

Histopathological review at Charing Cross Hospital demonstrated the classical features of a partial molar pregnancy including hydropic chorionic villi with irregular scalloped outlines, focal trophoblastic hyperplasia, and trophoblastic inclusions.

Genetic analysis of molar tissue

For four of eight informative STRs used in the first panel, the placental tissue was shown to be trisomic with three alleles of equal dosage while four further markers showed allelic imbalance with one allele present at twice the dosage of the other, confirming triploidy [Table 1]. For each of these markers the paternal contribution was present at a greater dosage than the maternal contribution consistent with a diandric triploid conceptus and confirming a diagnosis of partial hydatidiform mole. Finally the placental tissue was shown to have DNA from the X, but not the Y chromosome and was therefore female.

Centromeric markers, for which the paternal DNA sample was homozygous or where both parents shared the same genotype, were excluded from the analysis. For nine informative centromeric markers the molar genotype was consistent with a single allele from the patient and two contributions from the paternal DNA confirming a diandric triploid origin. For two loci the molar tissue was homozygous for an allele found in both parents. The paternal DNA was heterozygous for all eleven loci while the molar tissue was homozygous having only one of the two potential alleles from the father [Table 2].

Discussion

Assisted conception techniques are widely used in many countries and in the UK it is estimated that their use leads to approximately 2% of all current successful pregnancies. The introduction of a single sperm into an ovum in vitro by ICSI is now a standard technique for overcoming difficulties with sperm motility or the presence of only small numbers of functional sperm.

Table 1 Genotyping of partial molar pregnancy

Locus	Chromosome location	Genotype		
		Patient	Partial molar pregnancy	Partner
D1S1656	1q42	147[a]	139–147–155	139–155
D3S1764	3q23	230	226–230–234	226–234
F13A1[b]	6p25	288	284>288	284–292
D8S1110	8q11	268–281	268<272	256–272
D9S175	9q21	255–257	255<257	257–272
D10S179	10p15	114	112–114–134	112–134
D15S659	15q22	171–183	171–191–195	191–195
D16S753	16p11	265	257>265	257–261
AMEL	Xp22/Yp11	X	X	XY

[a]Genotypes are shown as allele sizes in base pairs

F13A1, coagulation factor XIII, A1 polypeptide; AMEL, amelogenin

Partial molar pregnancies are rare occurring in approximately 1 in 800 of conceptions and generally arise from two sperm concurrently entering and fertilizing the same ovum before the zona pellucida can form to prevent this process [5–7]. The ICSI procedure which places a single selected sperm into an ovum, would appear to be highly protective against the risk of having a partial mole and has been suggested as a procedure to avoid further molar pregnancies in women with two or more partial molar pregnancies [11]. Despite this, occasional cases of partial molar pregnancy have been described following assisted conception with ICSI and demonstrated to be triploid conceptions consistent with partial molar pregnancies [8, 9].

While the major cause of triploidy following ICSI is non-extrusion of the second polar body in the ovum, it cannot account for partial molar pregnancies that are triploids with two paternal contributions to the genome. There have been concerns that partial mole after ICSI could have arisen due to an additional sperm being inserted. However, it has been hypothesized that triploidy could also occur as a result of fertilization by a diploid sperm [8, 9] arising either by non-reduction or endoreduplication of the sperm DNA [12, 13]. While partial molar pregnancies generally arise by fertilization of an ovum by two sperm, occasional cases resulting from natural conception, in which the most likely origin of diandric triploidy is a diploid sperm, have been described [7]. In addition diploid sperm, although rare, are more common in infertile males [12].

In the case described here the histopathological diagnosis of partial molar pregnancy was confirmed by genetic analysis that demonstrated the molar DNA to be triploid with two paternal contributions to the genome. To further elucidate the origin of the additional paternal chromosome complement, a second panel of markers, located proximal to the centromeric regions of several autosomes, were used to genotype the parents and the molar tissue. In partial molar pregnancies that arise by dispermy, each of the sperm can potentially carry either of the two paternal alleles and the molar tissue is consequently equally likely to be homozygous or heterozygous for any marker for which the father is heterozygous. However, if the two

Table 2 Genotyping of partial molar pregnancy using markers for centromeric loci

Locus	Chromosome location	Genotype		
		Patient	Partial molar pregnancy	Partner
D1S252	1p13	97–107[a]	93>107	93–97
D2S139	2p12	122–124	103>122	103–108
D3S1271	3q12	90–96	94>96	94–98
D4S405	4p14	289–300	291>300	291–293
D5S426	5p13	316–322	316<324	304–324
D7S502	7q11	222–224	224	219–224
D8S1110	8q11	268–281	268<272	256–272
D16S753	16p11	265	257>265	257–261
D17S122	17p12	156–162	156	156–158
D18S57	18q12	87–99	83>87	83–91
D20S106	20q11	325–327	321>325	317–321

[a]Genotypes are shown as allele sizes in base pairs

paternal genomes arise from fertilization of an ovum by a diploid sperm, markers proximal to the centromere, that are unlikely to undergo recombination during meiosis, will be heterozygous if the chromosomes fail to divide during the first meiotic division or homozygous if the chromatids fail to divide during the second meiotic division. In the present case the molar tissue was female indicating failure of the first paternal meiotic division was unlikely. In addition, all paternally derived centromeric markers in the molar tissue were homozygous confirming the molar pregnancy had arisen following fertilization by a diploid sperm resulting from non-reduction at the second meiotic division.

Diploid sperm occur at approximately 0.2 % in healthy men and a higher levels of 1–2% in men with fertility problems [12] and, despite a perceived competitive disadvantage, these sperm are able to fertilize both naturally in vivo and when used in ICSI. It appears that diploid sperm may be difficult to recognize during this procedure and visualization of the pronuclear stage unremarkable in this particular setting with only two pronuclei visible in cases of partial molar pregnancy arising from diploid sperm as opposed to the three that would be visible in a typical partial molar pregnancy resulting from two separate sperm [13]. Demonstration that the partial molar pregnancy in this case was the result of fertilization with a diploid sperm may help to reassure women who have suffered a partial molar pregnancy after ICSI that this is not due to medical error. While diploid sperm are more common in infertile males, the incidence is still only 1–2% [12] and should not significantly reduce the chance of success in subsequent ICSI cycles.

References

1. Steptoe PC, Edwards RG. Birth after the reimplantation of a human embryo. Lancet. 1978;2:366.
2. Fertility treatment in 2010. http://www.hfea.gov.uk/docs/2011-11-16_Annual_Register_Figures_Report_final.pdf
3. Palermo G, Joris H, Devroey P, Van Steirteghem AC. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. Lancet. 1992;340:17–8.
4. Savage P, Williams J, Wong SL, Short D, Casalboni S, Catalano K, et al. The demographics of molar pregnancies in England and Wales from 2000 to 2009. J Reprod Med. 2010;55:341–5.
5. Lawler SD, Fisher RA, Pickthall VJ, Povey S, Evans MW. Genetic studies on hydatidiform moles. I. The origin of partial moles. Cancer Genet Cytogenet. 1982;5:309–20.
6. Jacobs PA, Szulman AE, Funkhouser J, Matsuura JS, Wilson CC. Human triploidy: relationship between parental origin of the additional haploid complement and development of partial hydatidiform mole. Ann Hum Genet. 1982;46:223–31.
7. Zaragoza MV, Surti U, Redline RW, Millie E, Chakravarti A, Hassold TJ. Parental origin and phenotype of triploidy in spontaneous abortions: predominance of diandry and association with the partial hydatidiform mole. Am J Hum Genet. 2000;66:1807–20.
8. Ulug U, Ciray NH, Tuzlali P, Bahçeci M. Partial hydatidiform mole following the transfer of single frozen-thawed embryo subsequent to ICSI. Reprod Biomed Online. 2004;9:442–6.
9. Dalmia R, Young P, Sunanda GV. A case of triploidy. Fertil Steril. 2005;83:462–3.
10. Fisher RA, Khatoon R, Paradinas FJ, Roberts AP, Newlands ES. Repetitive complete hydatidiform mole can be biparental in origin and either male or female. Hum Reprod. 2000;15:594–8.
11. Reubinoff BE, Lewin A, Verner M, Safran A, Schenker JG, Abeliovich D. Intracytoplasmic sperm injection combined with preimplantation genetic diagnosis for the prevention of recurrent gestational trophoblastic disease. Hum Reprod. 1997;12:805–8.
12. Egozcue S, Blanco J, Vidal F, Egozcue J. Diploid sperm and the origin of triploidy. Hum Reprod. 2002;17:5–7.
13. Rosenbusch BE. Mechanisms giving rise to triploid zygotes during assisted reproduction. Fertil Steril. 2008;90:49–55.