CASE REPORT

A pregnancy following PGD for X-linked autosomal dominant Incontinentia Pigmenti (Bloch-Sulzberger syndrome)

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Incontinentia Pigmenti (Bloch-Sulzberger syndrome) is a rare multisystem, ectodermal disorder associated with dermatological, dental and ocular features, and in <10% of cases, severe neurological deficit. Pedigree review suggests X-linked dominance with lethality in affected males. Presentation in female carriers is variable. Following genetic counselling, a mildly affected female carrier diagnosed in infancy with a de novo mutation was referred for preimplantation sexing, unusually selecting for male gender, with an acceptance of either normality or early miscarriage in an affected male. Following standard in-vitro fertilization and embryo biopsy, fluorescence in situ hybridization (FISH) unambiguously identified two male and two female embryos. A single 8-cell, grade 4 male embryo was replaced. A positive pregnancy test was reported 2 weeks after embryo transfer, although ultrasonography failed to demonstrate a viable pregnancy. Post abortive fetal tissue karyotyping diagnosed a male fetus with trisomy 16. This is an unusual report of preimplantation genetic diagnosis (PGD) being used for selection of males in an X-linked autosomal dominant disorder and demonstrates the value of PGD where amniocentesis or chorion villus sampling followed by abortion is not acceptable to the patient. This case also demonstrates the importance of follow-up prenatal diagnosis.

Key words: Incontinentia Pigmenti/preimplantation genetic diagnosis/sex selection/trisomy 16

Introduction

Incontinentia Pigmenti (IP) is a rare genodermatosis. It presents as a multisystem, ectodermal disorder accompanied by dermatological, dental and ocular features, and in some cases, severe neurological deficit. Clinical features of IP include typical cutaneous manifestations, although skin biopsy may be required for diagnostic confirmation. Skin lesions usually occur in infancy with blisters typically appearing at or soon after birth. In a study of 465 patients, Carney (1976) found that 30% of patients had notable neurological disease including convulsive disorders (13%), spastic paralysis (11%), motor retardation (7%), mental retardation (12%), and microcephaly (12%) (Carney, 1976).

The variable phenotype in affected females is consistent with random X chromosome inactivation. Pedigree review suggests X-linked dominance with lethality in males. This mode of inheritance is supported by the high female to male ratio, female to female transmission, the increased risk of miscarriage and by the occurrence of two reported cases of classical IP in males with Klinefelter syndrome (47,XXY) (Ormerod et al., 1987). Initially linkage to Xp11 was suggested following reports of five cases of females with de novo X; Xp11 translocations involving Xp11 in association with phenotypes similar to IP (Hodgson et al., 1985). However, using a large familial study, linkage to the Xq28 region was confirmed (Seifani et al., 1988, 1989).

Following appropriate genetic counselling, a mildly affected 29 year old nulliparous female carrier diagnosed in infancy with a de novo mutation was referred for preimplantation genetic diagnosis (PGD) for sexing of male gender, accepting the option of either normality, or early miscarriage in an affected male. A licence to proceed with PGD for this condition was obtained from the Human Fertilisation and Embryology Authority (HFEA, 1990).

Materials and methods

Luteal phase down regulation, ovarian stimulation, oocyte retrieval and ICSI were carried out as previously described (Muggleton Harris et al., 1993).

Embryo development and biopsy

The embryos were transferred to M3 medium (Medicult, UK) under oil at the 8-cell stage and biopsy was performed on day 3 of development. Blastomeres were assessed for the presence of nuclei prior to biopsy, and one blastomere with a distinct nucleus identified for removal from each embryo. Biopsy of a single cell was carried out as previously described (Ao et al., 1996).

Fluorescence in situ hybridization (FISH)

Slides were incubated in pepsin (100µg/ml 0.01N HCl; Sigma, Poole, Dorset, UK) for 20 min at 37°C. Following a brief wash in PBS and two washes in sterile water, slides were again dehydrated through an ethanol series. A probe mix (Vysis, Richmond, Surrey, UK) containing...
chromosome centromere-specific probes for X (directly labelled with Spectrum Green fluorochrome), Y (Spectrum Orange), and 18 (Spectrum Aqua) were used as previously described (Kuo et al., 1998). The presence of a single X signal, a single Y signal, and two signals corresponding to chromosome 18 was required for selection of male embryos.

**Tissue culture**

Post abortive chorionic villi retrieved at uterine evacuation were cleaned and washed in Ham’s F10 medium containing penicillin (100 units/ml), Streptomycin (100μg/ml) and gentamicin (10μg/ml). Cultures were established from explants and by enzyme dissociation with collagenase (Sigma, 1 mg/ml). Mitotic cells were collected from 7–15 day cultures and G-banded chromosome spreads prepared according to standard techniques.

**Results**

Oocyte retrieval was performed on day 15 of stimulation. A total of 10 cumulus-oocyte complexes was obtained and these yielded seven metaphase II oocytes suitable for injection with ejaculated spermatozoa, one germinal vesicle oocyte and a metaphase I egg. The metaphase I oocyte was inseminated with sperm for standard IVF in anticipation of late maturation. All seven oocytes remained intact following injection, and at 18 h, three oocytes showed distinct pronuclei and the metaphase I oocyte had also fertilized normally. By day 3 of development, two of the ICSI embryos and the IVF embryo had developed on to the 8-cell stage, while the other ICSI embryo remained at the 4-cell stage. All four embryos were morphologically of good quality with minimal fragmentation.

FISH analysis of chromosomes X, Y and 18 revealed unambiguous results from all four embryos. One ICSI embryo and the IVF embryo were clearly identified as male and the remaining two ICSI embryos as female. The HFEA Code of Practice (1998) at that time did not permit replacement of ICSI and IVF embryos within the same treatment cycle. Therefore a single male ICSI embryo was transferred to the uterus and the IVF male embryo cryopreserved. Luteal phase support was provided by 400 mg daily of progesterone pessaries (Cyclogest; Shire, Andover, UK) for 14 days.

The two female embryos were spread and tested with the same probe set; the biopsy diagnosis was confirmed.

A positive pregnancy test was reported 2 weeks later. Transvaginal ultrasonography at 6 weeks gestation revealed a singleton intrauterine pregnancy, with a small yolk sac and fetal pole, but no fetal heart [h human chorionic gonadotrophin (HCG) 11 747 IU/l]. A slowly rising βHCG of 40 324 IU performed at 7 weeks gestation and an ultrasound which failed to demonstrate fetal heart activity confirmed a non-viable pregnancy. Evacuation of retained products of conception yielded fetal tissue for karyotyping. A diagnosis of a male embryo was confirmed, but with primary trisomy 16 in each of two preparations from the cultured villi.

**Discussion**

In this case of IP, male gender was selected for transfer following PGD for this X-linked dominant condition, as specific molecular diagnosis of IP affected females in preimplantation embryos or by prenatal diagnosis was not yet possible. The risk of severe neurological deficit in females, although small, was unacceptable to this couple. The couple were prepared to accept a 50% risk of miscarriage of any males that implanted (i.e. miscarriage of those carrying the IP gene), and thus opted for either unaffected offspring, or spontaneous miscarriage. If a specific diagnostic test was possible, the phenotypic variation between female cases from mild to severe would be difficult to predict, and so would make any decision arising from prenatal diagnosis very difficult.

This couple requested PGD with transfer of male embryos to eliminate the possibility of an IP affected child. There are reports of requests for PGD for this disorder, conversely requesting transfer of female embryos, for couples who had suffered repeated miscarriage and were prepared to accept an IP affected female infant if further spontaneous abortions could be avoided (personal communication).

The transfer of embryos in this case presented a further dilemma. In a situation where 50% of those embryos that implant go on to miscarry, a case could be made for replacing more embryos than usual since replacing more embryos may improve the chance of a live birth following PGD treatment for IP. However in the UK replacement of more than three embryos is not permitted (HFEA Code of Practice, 1998). Furthermore, the HFEA legislation concerning mixed replacement of IVF and ICSI embryos in a single treatment cycle is a ruling that could have perhaps been relaxed in a case such as this. While we acknowledge the usefulness of segregation of IVF and ICSI embryos for essential follow up of ICSI pregnancies, it is unlikely that PGD pregnancies generally using ICSI should be considered as an equivalent cohort in the analysis. Since the time of this case mixed embryo transfers are now allowed in exceptional circumstances.

This case also demonstrates the importance of confirmation of the diagnosis made by PGD either by chorion villus sampling (CVS) or by amniocentesis, or as in the case of a non-viable pregnancy, by fetal tissue karyotyping. This is desirable not only to confirm the diagnosis but also to counsel the couple (Simpson and Liebaers, 1996). Before a PGD treatment cycle is commenced, and specifically once a diagnosis of pregnancy is made, the importance of confirmatory testing should be stressed to the patients. Should the pregnancy fail at any stage, follow-up by the diagnostic unit should be strongly recommended.

In this case, despite its frequency as a cause of spontaneous abortion, trisomy 16 was an unexpected finding; an IP affected male was considered to be the most likely reason for miscarriage. This finding not only demonstrates the importance of follow-up, but also suggests a role for aneuploidy screening of preimplantation embryos (Munné et al., 1995) as an adjunct to determining gender. In the only other reported case of PGD for IP, where a pregnancy was achieved, spontaneous abortion of a trisomy 9 fetus occurred (Munné et al., 1996). That paper also reported high levels of aneuploidy in those embryos; five out of seven (i.e. 71%) embryos were aneuploid if the trisomic abortus was included and 57% were aneuploid for chromosomes 13, 18 or 21. In our case, single blastomere...
biopsy and the use of 4-colour FISH to establish sex chromosome status and chromosome 18 copy number meant that screening of the blastomeres for other aneuploidies was not feasible technically, and aneuploidy screening is not offered at this centre for any couples undergoing PGD for X-linked disease. However, there is no reported association between IP and aneuploid offspring, and the published findings may therefore be incidental to the referral indication and in accord with the high incidence of chromosome aneuploidy reported in normally developing embryos (Harper et al., 1995; Delhanty et al., 1997). In our case, the two embryos diagnosed as normal diploid females on biopsy were confirmed as euploid on further FISH analysis of the entire embryo. Accumulating data on the frequency of different chromosome aneuploidies in early embryos (Handyside and Mackie Ogilvie, 1999) and development of more sophisticated and cheaper commercial probe panels may lead to routine aneuploidy screening in the future.

Despite a relatively small risk of severe neurological deficit in females, this couple’s decision to pursue male embryo selection demonstrates the value of PGD in cases where pregnancy termination following conventional prenatal diagnosis is not acceptable to the couple. In these cases, follow-up prenatal diagnosis for aneuploidy and confirmation of sex would almost certainly be similarly unacceptable. This case once more illustrates the importance of expert PGD counselling, both to explore and increase the couple’s understanding of the options available to them, and to emphasise the importance of karyotypic follow-up in the case of spontaneous pregnancy loss.

References


