Journal of Steroid Biochemistry & Molecular Biology 165 (2017) 124–130

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry & Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

Review

Preimplantation diagnosis and other modern methods for prenatal diagnosis

Joe Leigh Simpson[a,b,c,*], Svetlana Rechitsky[b,c]

[a] March of Dimes Foundation, 1275 Mamaroneck Avenue, White Plains, NY 10605, USA
[b] Florida International University, Herbert Wertheim College of Medicine, 11200 SW 8th Street, AHC2 667, USA
[c] Reproductive Genetics Innovation, 2910 MacArthur Blvd., Northbrook, IL 60062, USA

ARTICLE INFO

Article history:
Received 16 February 2016
Accepted 16 March 2016
Available online 21 April 2016

Keywords:
Preimplantation genetic diagnosis
Single gene disorders
Genotype
Aneuploidy

ABSTRACT

Prenatal treatment of congenital adrenal hyperplasia (CAH) has long involved prenatal treatment with dexamethasone, administered to the pregnant woman to prevent genital masculinization of an affected female fetus. Although it is unnecessary to treat unaffected or affected males because their genital development would not be disturbed, there has only been incremental progress in determining fetal gender sufficiently each to avoid treating males and unaffected females. Invasive procedures were initially necessary, with first-trimester amniocentesis at 15–20 weeks and then chorionic villus sampling (CVS) at 10–12 weeks gestation. Two approaches now allow personalized treatment of affected female fetuses prior to female genital differentiation. Only preimplantation genetic diagnosis (PGD) is available prior to clinical pregnancy. Recent technological advances have further allowed both single gene diagnosis (e.g., CAH) and aneuploidy detection concomitantly, resulting in far better pregnancy rates than heretofore possible in assisted reproduction technology.

Contents

1. Introduction and overview

For decades, the accepted protocol for prenatal treatment of congenital adrenal hyperplasia (CAH) has consisted of prenatal

* Corresponding author.
E-mail address: jsimpson@marchofdimes.org (J.L. Simpson).

http://dx.doi.org/10.1016/j.jsbmb.2016.03.022
0960-0760/

treatment with dexamethasone, administered to the mother to prevent genital masculinization in an affected female fetus [1,2]. Even though there is obviously no need to treat unaffected or affected males, whose genital development would not be disturbed, the only approach other than universal treatment of all at-risk women is to first determine fetal gender by an invasive procedure. Initially this was possible only with second-trimester amniocentesis at 15–20 weeks, meaning months of dexamethasone because genital differentiation occurs before the ninth week of gestation. In the 1980s chorionic villus sampling (CVS) permitted diagnosis at 10–12 weeks gestation. Administration of dexamethasone to the mother thus could be stopped earlier if female fetuses were unaffected or if the fetus were male (albeit to be resumed if affected after birth). Although CVS was an advance in terms of early prenatal diagnosis, treatment still had to begin before fetal gender and genotype were known. Two approaches now allow personalized treatment prior to genital differentiation. Diagnosis by cell free DNA analysis is discussed in Chapter __. In this chapter we shall discuss preimplantation genetic diagnosis (PGD).

2. Development of preimplantation genetic diagnosis (PGD)

Preimplantation genetic diagnosis (PGD) is well established as an integral component of the prenatal genetic diagnosis armamentarium [3–6]. This approach is the only way to accommodate a couple wishing to avoid clinical termination of pregnancy. Genotype can be determined before implantation in a couple at risk for CAH, unequivocally identifying not only gender but affected from unaffected embryos.

Although often considered a relatively recent advance in prenatal genetic diagnosis (PGD), PGD was actually envisioned in 1968. Gardner and Edwards [7] biopsied a rabbit blastocyst and performed X-chromatin analysis, realizing application for X-linked recessive traits. Harper [3] provides a history of the development of PGD thereafter. Availability of polymerase chain reaction (PCR) finally made PGD practical in humans. In Europe, emphasis focused on blastomere biopsy of the 3 day cleavage stage embryos (6–8 cells), whereas in the United States polar body biopsy was initially pursued. In 1990, sex was determined in United Kingdom in a pregnancy at risk for X-linked recessive ornithine transcarbamylase deficiency (OTC) [8]. This was followed by detection of cystic fibrosis, using nested primer PCR [9]. In the United States, Verlinsky et al. used polar body biopsy to achieve a single gene diagnosis in 1987 [10], but the first full-length peer publication was not until 1990: α 1-antitrypsion deficiency [11]. Polar body biopsy for cystic fibrosis was soon reported by the same U.S. group [12].

Detecting chromosomal abnormalities became possible with development of fluorescence in situ hybridization (FISH) using chromosome specific probes [13,14]. Pregnancy following embryo biopsy subjected to X and Y FISH was reported by Grifo et al. [15], setting the stage for modern PGD aneuploidy testing [16]. As will be discussed, PGD aneuploidy testing will be increasingly expected to accompany PGD for 21-hydroxylase deficiency (CAH) and other single gene disorders.

2.1. Obtaining cells for PGD

DNA is required for PGD. There are three approaches, two already mentioned: (1) polar body biopsy, utilizing female gametes (oocytes); (2) blastomere biopsy, utilizing the 3-day, 6- to 8-cell cleavage stage embryo; and (3) trophectoderm biopsy, utilizing the 5- to 6-day blastocyst that contains approximately 120 cells. In this section we shall briefly describe approaches and relative benefits for each. Extensive details and illustrations are provided in atlas form by our group [6].

2.2. Polar body

Oocyte genotype can be deduced on the basis of the first and second polar bodies [5,6]. In PGD for CAH, the underlying principle would be that the first polar body from a heterozygous individual (carrier mother) showing a mutant maternal allele should be complemented by a primary oocyte having the normal allele. Oocytes thus deduced to be genetically normal can be fertilized in vitro and transferred for potential implantation. A genetically normal polar body conversely indicates a genetically abnormal oocyte; thus, fertilization would not proceed. If the assessment were for aneuploidy to accompany CAH, a polar body with a chromosome number other than 23 (i.e., 22 or 24) would signal by deduction that the oocyte is not suitable for fertilization because it must be trisomic or monosomic, respectively. The same principle would apply with analysis of the second polar body.

A unique advantage of first polar body biopsy is that this can occur before fertilization; thus, analysis offers the unique possibility of *preconceptional* diagnosis. For certain couples this is the only acceptable form of prenatal diagnosis because only genetically normal oocytes need be fertilized. Reasonable pregnancy rates can be achieved even if regulations were to require all fertilized oocytes be transferred. By contrast, the second polar body is not extruded until the oocyte is fertilized by sperm; thus, criteria for pre-conceptional biopsy would not be met.

In single gene diagnosis, one must take into account recombination. Crossing-over is required between homologous chromosomes, almost obligatory for proper segregation of homologous chromosomes in meiosis I. If recombination involving sister chromatids were not to occur, the two chromatids of a single chromosome would in the first polar body be identical in genotype and exactly complementary to the oocyte containing the homologous chromosome; the second polar body (chromatid only) would thus be identical in genotype to the oocyte. This is the scenario illustrated above. However, if crossing over were to involve the chromosomal region containing the gene in question (e.g., chromosome 6 and the 21-hydroxylase locus), the single chromosome in the first polar body would show a different allele on each of its two chromatids (heterozygosity). The genotype of the segregated oocyte could then not be predicted; biopsy of the second polar body would be necessary. In practice, both first and second polar bodies are biopsied in almost all centers.

The obvious *dis*advantage of polar body biopsy is inability to assess *paternal* genotype, obviously precluding application if a paternal mutation must be sought. In testing for single-gene autosomal recessive disorders like 21-hydraxylase CAH, there would be loss of efficiency if the paternal genotype transmitted to the fetus could not be taken into account. Transmission of a normal allele by the mother would, however, mean the fetus was at least heterozygous (unaffected). Demonstration of the normal maternal locus would exclude an affected embryo.

2.3. Cleavage stage embryo

Until approximately 5 years ago, most PGD cases were performed by blastomere biopsy of the cleavage state embryo. The zona pellucida – a glycoprotein layer that surrounds the cleavage stage embryo – is breached by mechanical, laser, or chemical means to extract a cell containing DNA (blastomere). Only a single cell is typically removed because even one fewer cell at this stage is believed to reduce embryo survival as much as 10%. Reduction of survival of this magnitude is derived from data correlating pregnancy rates with numbers of blastomeres remaining after thawing cryopreserved embryos [17]. Removal of two cells reduces the pregnancy rate considerably more [18]. One