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(54) **Title:** IDENTIFYING CHROMOSOMAL ABNORMALITIES IN CELLS OBTAINED FROM FOLLICULAR FLUID

(57) **Abstract:** The present disclosure describes methods for identifying chromosomal abnormalities in cells obtained from follicular fluid to identify, for example, gonadal mosaicism or low-grade gonadal mosaicism. The chromosomal abnormalities are identified using genetic analysis that can detect chromosomal, DNA or gene expression abnormalities in the cells.

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IDENTIFYING CHROMOSOMAL ABNORMALITIES IN CELLS OBTAINED FROM FOLLICULAR FLUID

CROSS REFERENCE TO RELATED APPLICATION

This application claims priority from Indian provisional patent application filed on 11th June 2004, under Application No. 642/MUM/2004.

FIELD OF THE INVENTION

The present disclosure relates to a method for identification of chromosomal abnormalities in an individual, including gonadal mosaicism, in cells obtained from follicular fluid.

DESCRIPTION OF RELATED ART

Chromosomes, which are present in the nucleus of eukaryotic cells, carry the genetic information of a cell. In humans, there are normally 23 pairs of chromosomes in each diploid cell for a total of 46 chromosomes. Deviations in chromosome structure or number may lead to developmental and physiological abnormalities. Structural chromosome abnormalities may be translocations (reciprocal and Robertsonian), deletions, inversions (paracentric and pericentric), ring chromosomes, and isochromosomes. Structural chromosome abnormalities also include rearrangement of chromosomes, resulting from breakage of a chromosome and subsequent reunion in a different configuration which may be either balanced or unbalanced (Mueller RF and Young ID; Emery's Elements of Medical Genetics Ninth Edition, 1995, p. 37).

Numerical chromosome abnormalities are the loss or gain of one or more chromosomes (aneuploidy), the loss of an entire set of chromosomes (monoploidy), or the gain of one or more complete sets of chromosomes (polyploidy). Aneuploidy may occur in the form of nullisomy (the loss of a pair of homologous chromosomes, $2n$-2), monosomy (the loss of a single chromosome, $2n$-1), trisomy (the gain of a single chromosome, $2n$+1), or

tetrasomy (the gain of a pair of homologs, $2n+2$). Aneuploidy may occur as a result of non-disjunction, which is when paired chromosomes fail to separate during cell division. If non-disjunction occurs during gametogenesis, either half (non-disjunction during meiosis II) or all (non-disjuction during meiosis I) of the resulting gametes will be aneuploid. Non-disjunction may also occur during mitosis, producing two cell lines with different numbers of chromosomes.

Consequently, a single individual might have two or more cell populations with distinct karyotypes or genetic makeup, which may be referred to as mosaicism, chromosomal mosaicism, or gonadal mosaicism. A mosaic as used herein is an organism composed of cells with two or more different genotypes. If the non-disjunction event occurs early in development of an individual, different parts of the individual's body may have cells with different total chromosome numbers or chromosomal content. Mosaicism may also result from gene mutation events. Similarly, if a mutation occurs early in embryonic growth, some, but not all, of the individual's cells may have the mutation.

Chromosomal mosaicism can occur in any number of tissues, depending on the timing of the non-disjunction event and the cell type where it occurs. Consequently, it is difficult to predict the type or severity of symptoms in an individual with chromosomal mosaicism. When mosaicism is restricted to gonads it is known as gonadal mosaicism (Goldstein *et al.*, J. Pediatrics, 1977, 90:604). Gonadal mosaicism may arise due to a mutation occurring in the gonads of a developing fetus.

Chromosomal abnormalities are also known to affect ovarian development and function depending on the percentage of abnormal cells in the ovaries (Cunniff *et al.*, Hum Genet., 1991, 86:553-556). For example, women missing a copy of the X chromosome (45,X) or having an additional copy of the X chromosome (46,XX) have been shown to be at an increased risk of premature ovarian failure (Cunniff *et al.*, Hum. Genet., 1991, 86:553-556; Zinn *et al.*, Trends Genet. 1993, 9:90-93). Studies have shown that as high as 99%

of 45,X monosomy conceptions result in early miscarriages (Hook and Warburton, Hum. Genet., 1983, 64: 24-27; Hassold *et al.*, Am. J. Hum. Genet., 1988, 42:534-541). Mosaic X-chromosome monosomy (45,X/46,XX) is associated with follicular artesia, gonadal dysgenesis, and is also a common cause for early pregnancy loss (Zinn *et al.*, Trends Genet., 1993, 9:90-93; Shreck *et al.*, Emery and Rimoni's Principles of Practice of Medical Genetics, Vol. 1, Churchill Livingstone, 2002, 982-97).

Females with gonadal mosaicism may have chromosomally abnormal oocytes, which can cause infertility or chromosomal abnormalities in the developing fetus. These possible effects warrant counseling or treatment options like ooctye/embryo donation, pre-implantation genetic diagnosis, or prenatal diagnosis. Consequently, improvements in the identification or diagnosis of gonadal mosaicism will improve patient management.

Current approaches of identifying or diagnosing chromosomal abnormalities are limited for gonadal mosaicism, and the available techniques are invasive or inefficient for detecting low-grade mosaicism. For example, when a chromosomal abnormality is suspected in an individual, it may be confirmed or ruled out through analysis of a blood sample. This approach, however, may not necessarily detect mosaicism restricted to one organ or tissue or even a limited proportion of cells in a given tissue. For example, blood sample analysis may not detect gonadal mosaicism. Moreover, individuals with gonadal mosaicism may remain largely asymptomatic throughout life, further lessening the chances of diagnosis.

To diagnose mosaicism restricted to a single tissue, cells from that specific tissue must be analyzed. Detection of mosaicism in a given tissue depends on the number of cells affected and the number of cells analyzed. Karyotyping is a frequently used genetic test for chromosomal analysis whereby the complete chromosomal constitution of a cell of an individual is examined by imaging the chromosomes and counting the chromosomes. Because chromosomes are best visualized in the metaphase stage of cell division, cells

subjected to karyotyping are preferably cultured and arrested at the metaphase stage of cell division. This makes karyotyping a time consuming and tedious procedure and does not allow for analysis of very large numbers of cells. Consequently, karyotyping may fail to identify low-grade mosaicism in which only a small proportion of cells in an organ or tissue are affected.

Presently, to detect gonadal mosaicism, gonadal tissue is isolated by gonadal biopsy. Gonadal biopsy is not a routine procedure and is performed by fine needle aspiration biopsy or wedge biopsy obtained either by laparotomy or laparoscopy. The obtained tissue sample is then karyotyped, which may fail to identify low-grade gonadal mosaicism. Consequently, current approaches to the detection of chromosomal abnormalities have a limited ability to detect gonadal mosaicism, particularly low-grade gonadal mosaicism.

Therefore, it is desirable to provide a minimally-invasive method for identifying chromosomal abnormalities, including gonadal mosaicism, which preferably can identify even low-grade gonadal mosaicism.

BRIEF SUMMARY OF THE INVENTION

The present disclosure is directed to methods of identifying at least one chromosome abnormality in cells obtained from follicular fluid comprising:

a) obtaining cells from follicular fluid;

b) subjecting the cells to genetic analysis;

wherein a portion of the cells have at least one chromosome abnormality. In certain embodiments, the present disclosure is directed to methods of identifying reproductive system abnormalities, wherein the identification of at least one chromosomal abnormality in a portion of the cells obtained from follicular fluid is indicative of a reproductive system abnormality. Other embodiments of the present disclosure are directed to

methods of identifying gonadal mosaicism by identifying at least one chromosomal abnormality in cells obtained from follicular fluid using these same methods. In certain embodiments the gonadal mosaicism is low-grade gonadal mosaicism, wherein preferably less than 10% of the cells, more preferably less than 10% to 5% of the cells in a tissue or organ have a chromosome abnormality. In preferred embodiments the follicular fluid is obtained from an animal, more preferably a human. The cells obtained from follicular fluid may arise from reproductive tissues, and in certain embodiments are somatic cells. In preferred embodiments, the follicular fluid is obtained during an *in vitro* fertilization procedure or an intracytoplasmic sperm injection procedure. In other embodiments, the follicular fluid is not obtained in connection with either of these procedures. Preferably the follicular fluid is obtained from one or both ovaries of a subject.

The present disclosure is also directed to methods for assaying an increased risk of infertility in an animal comprising:

a) obtaining cells from follicular fluid of the animal; and

b) subjecting the cells to genetic analysis,

wherein the identification of at least one chromosomal abnormality in a portion of the cells is indicative of an increased risk of infertility. In preferred embodiments the animal is a human. The cells obtained from the follicular fluid may arise from reproductive tissues, and in certain embodiments are somatic cells. In preferred embodiments, the follicular fluid is obtained during an *in vitro* fertilization procedure or an intracytoplasmic sperm injection procedure. In other embodiments, the follicular fluid is not obtained in connection with either of these procedures. Preferably the follicular fluid is obtained from one or both ovaries of the animal. In preferred embodiments, when an animal is identified as having an increased risk of infertility, and the animal is undergoing an *in vitro* fertilization procedure, embryos resulting from oocytes isolated from the animal may be subjected to pre-implantation genetic analysis. In more preferred embodiments, when the genetic analysis demonstrates that an embryo does not have a chromosome abnormality, the embryo is preferably transferred into the animal's uterus.

Any of the methods disclosed herein may use genetic analysis methods that are well known to those of skill in the art to identify chromosome abnormalities in cells obtained from follicular fluid. In preferred embodiments, the genetic analysis is fluorescent *in situ* hybridization, karyotyping, or DNA sequencing. In other preferred embodiments, the genetic analysis is comparative genome hybridization (CGH) (*e.g.*, performed with metaphase chromosomes or a CGH-array), multicolor-banding (MCB), quantitative FISH (Q-FISH), polymerase chain reaction (PCR), genetic bit analysis (GBA), multiplex sequencing, SNaPshot, MassEXTEND, MassArray, microarray ligation, microarray miniseq, tag arrays, arrayed primer extention (APEX), microarray primer extension, GOOD assay, coded microspheres, restriction fragment length polymorphism analysis (RFLP), allele specific oligonucleotide (ASO) analysis, methylation-specific PCR (MSPCR), pyrosequencing analysis, acycloprime analysis, reverse dot blot, GeneChip microarrays, dynamic allele-specific hybridization (DASH), peptide nucleic acid (PNA) and locked nucleic acids (LNA) probes, TaqMan, Molecular Beacons, intercalating dye, FRET primers, AlphaScreen, SNPstream, Invader assay, Template-directed incorporation (TDI), fluorescence polarization, sequence-coded oligonucleotide ligation assays, ligase chain reaction, padlock probes, rolling circle amplification, or colorimetric oligonucleotide ligation assay (OLA).

Any number of chromosome abnormalities may be identified in the cells isolated from follicular fluid, and may be indicative of gonadal mosaicism or low-grade gonadal mosaicism. In certain embodiments, the chromosome abnormality identified in all or a portion of the cells from the follicular fluid is aneuploidy, a translocation, a deletion, a microdeletion, an inversion, or a duplication. The identified aneuploidy may be complete or partial trisomy, for example trisomy 13, trisomy 16, trisomy 18, trisomy 21, trisomy 22, XXY, XYY, or XXX; complete or partial monosomy, for example monosomy X, monosomy 13, monosomy 16, monosomy 18, monosomy 21, or monosomy 22; or complete or partial nullisomy, for example for chromosome 13, 16, 18, 21, 22, X, or Y.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present disclosure. The disclosure may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

Figure 1. Photomicrograph of FISH analysis of a normal, diploid human female cell obtained from follicular fluid. Probes specific for chromosomes X (green), Y (orange), and 18 (aqua) were used and the cells were counterstained with DAPI dark blue. Two aqua signals for chromosome 18 and two green signals for chromosome X are seen, indicating a normal, diploid female cell.

Figure 2. Photomicrograph of FISH analysis of a monosomy 18 cell obtained from the follicular fluid of a female human. Probes specific for chromosomes X (green), Y (orange), and 18 (aqua) were used and the cells were counterstained with DAPI dark blue. One aqua signal for chromosome 18 and two green signals for chromosome X are seen, indicating monosomy 18 in the female cell.

Figure 3. Photomicrograph of FISH analysis of a monosomy X cell obtained from the follicular fluid of a female human. Probes specific for chromosomes X (green), Y (orange), and 18 (aqua) were used and the cells were counterstained with DAPI dark blue. Two aqua signals for chromosome 18 and one green signal for chromosome X are seen, indicating monosomy X in the female cell.

Figure 4. Photomicrograph of FISH analysis of a trisomy 18 cell obtained from the follicular fluid of a female human. Probes specific for chromosomes X (green), Y (orange), and 18 (aqua) were used and the cells were counterstained with DAPI dark blue. Three aqua signals for chromosome 18 and two green signals for chromosome X are seen, indicating trisomy 18 in the female cell.

Figure 5. Photomicrograph of FISH analysis of a trisomy X cell obtained from the follicular fluid of a female human. Probes specific for chromosomes X (green), Y (orange), and 18 (aqua) were used and the cells were counterstained with DAPI dark

blue. Two aqua signals for chromosome 18 and three green signals for chromosome X are seen, indicating trisomy X in the female cell.

Figure 6. Photomicrograph of FISH analysis of a normal cell obtained from the follicular fluid of a female human. Probes specific for chromosomes 13 (green) and 21 (orange) were used and the cells were counterstained with DAPI dark blue. Two green signals for chromosome 13 and two orange signals for chromosome 21 are seen, indicating a diploid cell with the normal complement of chromosomes 13 and 21.

Figure 7. Photomicrograph of FISH analysis of a trisomy 13 cell obtained from the follicular fluid of a female human. Probes specific for chromosomes 13 (green) and 21 (orange) were used and the cells were counterstained with DAPI dark blue. Three green signals for chromosome 13 and two orange signals for chromosome 21 are seen, indicating a the female cell is trisomy 13.

Figure 8. Photomicrograph of FISH analysis of a trisomy 21 cell obtained from the follicular fluid of a female human. Probes specific for chromosomes 13 (green) and 21 (orange) were used and the cells were counterstained with DAPI dark blue. Two green signals for chromosome 13 and three orange signals for chromosome 21 are seen, indicating the female cell is trisomy 21.

Figure 9. Karyotype analysis of a monosomy X cell obtained from the follicular fluid of a female human. A single X chromosome is seen in this karyotype, indicating this cell is monosomy X. In analysis of additional cells from the same follicular fluid sample, 3 of the 20 cells analyzed displayed a similar pattern, suggesting gonadal mosaicism for monosomy X.

Figure 10. Karyotype analysis of a trisomy 21 cell obtained from the follicular fluid of a female human. Three 21 chromosomes are shown in this karyotype, indicating this cell is trisomy 21.

DETAILED DESCRIPTION OF THE INVENTION

The present disclosure describes methods of identifying chromosomal abnormalities in a subject by obtaining cells from follicular fluid and analyzing those cells, for example by

genetic analysis, for chromosomal abnormalities. Preferably, the disclosed method identifies subjects with gonadal mosaicism or low-grade gonadal mosaicism. Low-grade gonadal mosaicism is when a subject is mosaic for preferably less than 10% of the cells, and more preferably less than 5% to 1% of the cells, in a tissue or organ. In one embodiment, the cells analyzed by the methods disclosed herein arise from reproductive tissue of the subject from whom the follicular fluid sample was isolated. In another embodiment, the cells analyzed by the methods disclosed herein are somatic cells of the subject from whom the follicular fluid sample was isolated, and exclude any gamete cells such as oocytes present in the follicular fluid. Chromosomal abnormalities may be identified using any one of a number of genetic analysis methods known to those of skill in the art. The phrase "genetic analysis" as used herein refers to any chromosome, DNA, or RNA based analysis which can detect chromosomal, DNA or gene expression abnormalities in cells of an individual, or preferably, in cells obtained from follicular fluid.

Cells obtained from follicular fluid may also be subjected to DNA analysis in order to identify single gene disorders, rather than chromosomal level abnormalities. The identification of single gene disorders, imprinting disorders, or predisposition to cancer can be accomplished using any method suitable for identification of at least one nucleic acid substitution, for example, a single nucleotide polymorphism (SNP). While the above enumerated genetic analysis techniques are preferred, other methodologies capable of employing cells from follicular fluid for identifying chromosomal abnormalities including gonadal mosaicism or low-grade mosaicism are well within the scope of the present disclosure and the knowledge of one of skill in the art.

Chromosome abnormalities are a common cause of infertility and congenital birth defects, and include both structural and numerical abnormalities. An organism may have mosaicism when chromosomal abnormalities are found only in particular cell populations or lineages in an organism while other cells are normal. While an entire organism may be mosaic, mosaicism may also be restricted to particular organs or tissues, as in gonadal

mosaicism. Gonadal mosaicism may remain undetected because affected individuals may be largely asymptomatic. However, when gonadal mosaicism is a suspected cause of infertility, current methods for identification or detection of gonadal mosaicism require a surgical procedure for collection of a tissue sample for analysis. Thus, the current analytical techniques are time consuming and limit the number of cells that may be analyzed, thereby limiting the detection of low-grade gonadal mosaicism.

The present disclosure analyzes cells obtained from follicular fluid by genetic analysis to detect chromosomal abnormalities, and for diagnosing gonadal mosaicism, for example low-grade gonadal mosaicism. For example, follicular fluid is isolated from a patient undergoing oocyte collection for *in vitro* fertilization. In this procedure, the follicular fluid containing the oocytes is aspirated from the patient's ovaries, using for example transvaginal ultrasound. The oocytes, with the surrounding cumulus cells, are identified and removed from the follicular fluid and the fluid is then usually discarded. Consequently, for patients already undergoing oocyte retrieval, the present disclosure requires no additional surgical procedures to enable detection of gonadal mosaicism.

In a preferred embodiment follicular fluid is collected during oocyte retrieval for *in vitro* fertilization. Procedures for follicular aspiration are well known to those of skill in the art of gynecology and obstetrics. Generally, oocytes are retrieved by follicular aspiration 36 hours after induction of ovulation, after which the oocytes, with their surrounding cells, are removed from the excess follicular fluid. A preferable procedure for obtaining cells from the remaining follicular fluid is centrifugation, but any known technique of cell separation may be used. Preferably, the follicular fluid is centrifuged for any length of time from about 1 minute to about 30 minutes, at about 1000 rpm to 3000 rpm and at any temperature from about 20°C to about 40°C. In an alternative embodiment, the follicular fluid is not collected during oocyte retrieval for *in vitro* fertilization, for example by follicular aspiration or laparoscopy.

Once the cells have been separated from the follicular fluid, they may be subjected to genetic analysis for the detection of chromosomal abnormalities. In a preferred embodiment, the cells are subjected to FISH analysis. For example, the cells are subjected to treatment with hypotonic solution for approximately 5 to 45 minutes at a temperature of about 20°C to about 40°C and then fixed on a suitable surface with a suitable fixative, such as Carnoy's fixative, at about 1°C. to about 25°C. Once fixed, appropriate probes may be hybridized with the sample cells, using methods known to those skilled in the art. Preferably, labeled DNA probes for the chromosomes to be analyzed are premixed with blocking DNA and hybridization buffer and then applied to the fixed cells from the follicular fluid sample. The probe and sample are then denatured for about 5 minutes at about 73°C. and incubated to allow hybridization in a dark hybridization chamber at about 37°C. for about 16 to 18 hours. Following hybridization, the slides are washed for about 2 minutes at about 73°C. in a solution of 1 mL 20 X SSC, 49 mL RO H_2O, and 150 µl Igepal or other similar surfactant, and then for about 1 minutes at about room temperature in a solution of 5 mL 20 X SSC, 45 mL RO H_2O, and 50 µl Igepal or other similar surfactant. After rinsing, the slides are allowed to dry and counterstained using, preferably, DAPI. The cells may then be analyzed using a fluorescence microscope. Using these preferred methods, about 20 to 1000 cells may be analyzed from a given sample of follicular fluid. Because the FISH approach allows analysis of a large number of cells from a single follicular fluid sample, low-grade gonadal mosaicism, affecting only a small proportion of cells in the ovaries, may be detected.

In preferred embodiments, cells isolated from follicular fluid are analyzed for chromosome abnormalities, for example aneuploidy, such as nullisomy, monosomy or trisomy, of any chromosome may be detected using genetic analysis methods well known to those of skill in the art. In preferred embodiments, cells isolated from follicular fluid are analyzed to determine whether they are aneuploidy for chromosomes 13, 15, 16, 18, 21, 22, X or Y. Chromosome abnormalities may be detected using FISH analysis with probes specific for those chromosomes. Similarly, detecting cells that are monosomy for

chromosomes 13, 15, 16, 18, 21, 22, X or Y may be performed using probes specific for those chromosomes. Monosomy of chromosomes 15, 16, 21, and 22 are known to be involved in pregnancy miscarriage (Munne, S. *et al.*, 2004, Reprod. Biomed. Online, 8:91-90).

In other preferred embodiments, karyotyping may be used to analyze chromosome abnormalities in the cells. In a preferred procedure for karyotyping, cells obtained from follicular fluid are first cultured using suitable culture media. Examples of commercially available culture media include Amniomax, F10 medium, or Rose Park Memorial Institute medium. The cells are preferably cultured using standard techniques known to those skilled in the art at a favorable temperature (*e.g.*, 37° C.) until confluent growth is obtained. Next, the cultured cells are preferably harvested using conventional methods and fixed on an appropriate surface with a suitable fixative, such as Carnoy's fixative, at a temperature of about 1°C. to about 25°C. Banding of the slides is performed using methods known to those of ordinary skill in the art, and the slides are analyzed for metaphase chromosomes under an appropriate microscope. Using these preferred methods, approximately 1 to 40 cells may be analyzed from a given sample of follicular fluid.

In other preferred embodiments, comparative genome hybridization (CGH) is used to identify chromosome abnormalities, including subtle chromosomal abnormalities. CGH is based on quantitative two-color FISH, using probes synthesized from the test sample, for example cells isolated from follicular fluid, and a normal, control reference sample. Preferably, the probes are simultaneously hybridized to metaphase chromosomes from a normal reference sample. Comparison of the signal intensity from the hybridized probes may indicate regions of abnormality in the follicular fluid cells.

In other embodiments, the probes constructed above may be applied to a DNA array in a technique called CGH-array. CGH-array has been shown to confirm abnormalities such

as mosaicism of trisomy 20 (Schaeffer *et al.*, Am. J. Hum. Genet., 2004; 74:1168-1174), unbalanced translocation (Klein *et al.*, Clin. Genet., 2004; 65:477-82), unbalanced subtelomeric rearrangements (Ness *et al.*, Am. J. Med. Genet, 2002, 113:125-136), and unbalanced inversions or chromosomal rearrangements (Daniely *et al.*, Cytogenet. Cell. Genet., 1999, 86:51-5). Methods for CGH array and preparing chromosome specific DNA libraries are described and known in the art (Hu, *et al.*, Mol. Hum. Reprod., 2004, 10:283-289; Bolzer *et al.*, Cytogenet. Cell. Genet., 1999, 84:233-240). Using array-based CGH for determining genetic mosaicism in a cell population also has been disclosed by Mansoor Mohammed in published United States Patent Application No. 20030124584, which is herein incorporated by reference in its entirety.

Other methods well know to those of skill in the art for analyzing chromosomal and genetic abnormalities include, but are not limited to, multicolor-banding (MCB), quantitative FISH (Q-FISH), polymerase chain reaction (PCR), genetic bit analysis (GBA), multiplex sequencing, SNaPshot, MassEXTEND, MassArray, microarray ligation, microarray miniseq, tag arrays, arrayed primer extension (APEX), microarray primer extension, GOOD assay, coded microspheres, restriction fragment length polymorphism analysis (RFLP), allele specific oligonucleotide (ASO) analysis, methylation-specific PCR (MSPCR), pyrosequencing analysis, acycloprime analysis, reverse dot blot, GeneChip microarrays, dynamic allele-specific hybridization (DASH), peptide nucleic acid (PNA) and locked nucleic acid (LNA) probes, TaqMan, Molecular Beacons, intercalating dye, FRET primers, AlphaScreen, SNPstream, Invader assay, Template-directed incorporation (TDI), fluorescence polarization, sequence-coded oligonucleotide ligation assays, ligase chain reaction, padlock probes, rolling circle amplification, and colorimetric oligonucleotide ligation assay (OLA).

In *in vitro* fertilization programs, pre-implantation genetic diagnosis of oocytes and embryos has become the technique of choice to select against abnormal embryos before embryo transfer. Thus, in alternative embodiments, in subjects identified as having gonadal mosaicism, pre-implantation genetic diagnosis and the diagnosis of chromosome

abnormalities in oocytes and embryos may be performed to increase the probability of selecting and implanting an embryo which will survive through gestation.

Finally, the identification of chromosome abnormality, including gonadal mosaicism, is applicable in other animals, preferably mammals, and particularly transgenic animals. Genetic mosaicism occurs frequently in transgenic animals produced by pronuclear microinjection. A successful method of screening founder animals for germline mosaicism prior to mating may reduce costs of propagating transgenic lines and improve the efficiency of transgenic animal production by screening founder animals for germline mosaicism prior to mating for the production of transgenic mammals.

The methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the methods of this disclosure have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the methods and in the steps or in the sequence of steps of the methods described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents that are chemically or physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific

embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Example 1

1) Collection and Processing of Cells from Follicular Fluid Samples

Leftover follicular fluid samples from patients undergoing *in vitro* fertilization were collected in centrifuge tubes in approximately 5 to 10 mL aliquots. The samples were then centrifuged at about 1000 rpm for 10 minutes. Most of the supernatant was then removed, leaving behind approximately 0.5 mL of fluid above the pellet. About 8 mL of 75 mM KCl, pre-warmed to 37°C., was added to the pellet, mixed, and then incubated at 37°C. for 20 minutes. After incubation, the tubes were centrifuged at about 1000 rpm for 10 minutes. The supernatant was then discarded, leaving behind approximately 0.5 mL of fluid above the pellet. About 8 mL of cold Carnoy's fixative was added while constantly mixing. The tubes were incubated at 2-8° C. for at least 30 minutes and then centrifuged at 1000 rpm for 10 minutes. After centrifugation, the supernatant was discarded, leaving behind approximately 0.5 mL of fluid above the pellet. The wash step was repeated 2 times with the fixative.

2) Slide preparation and FISH analysis

One or two small squares were marked with a diamond marker on the backside of clean slides. 10 µl of fixed cells were added to each square and the slide was allowed to air dry. The slides were serially dehydrated in alcohol: 70%, one minute; 85%, one minute; 100%, two minutes). Next, coverslips were cut to the size of the sample area, and 1-2 µl of probe was applied to the coverslip. The slide was inverted on the coverslip and sealed with rubber cement. The slide was then denatured by placing it on a slide warmer at 73°C. for five minutes. The slides were then immediately placed inside a hybridization box and incubated at 37°C for 16 to 18 hours. After incubation, the coverslips were removed in 5 mL of 20 X SSC and 45 mL of RO H_2O. The slide was then washed with 1 mL 20 x SSC and 49 mL of RO H_2O and 150 µl Igepal (Sigma) for two minutes, followed by another wash in a solution of 5 mL 20 X SSC, 45 mL H_2O, and 50 µl Igepal

for one minute. The slide was air dried and mounted with 10 µl mounting medium (200 ng/mL DAPI in Vectashield, Vector Lab) and placed on a coverslip The slide was sealed with rubber cement, and were viewed under a fluorescent microscope.

3) Probes for FISH analysis

For detecting gonadal mosaicism for chromosomes 18, X, and Y, the probe cocktail used contained CEP 18 Spectrum Aqua / X Spectrum Green / Y Spectrum Orange (AneuVysion EC DNA probe kit, Vysis). Although used as a cocktail, the individual probes for the 18, X and Y chromosomes may also be used separately. The experimental results of these analyses are shown below in Table 1:

Table 1

Sr. No.	Sample No.	One Ovary				Conception Status
		No. of cells analyzed	Normal for X & 18 (%)	X chr. abn. %	chr. 18 abn. %	
1	FF-001	100	100	0	0	-
2	FF-002	100	96	2	2	-
3	FF-003	200	90	10	0	-
4	FF-004	100	100	0	0	-
5	FF-005	100	100	0	0	-
6	FF-006	100	100	0	0	+
7	FF-007	100	100	0	0	+
8	FF-008	100	100	0	0	-
9	FF-009	100	95	2	3	-
10	FF-010	100	95	5	0	-
11	FF-011	100	92	3	5	-
12	FF-012	100	100	0	0	-

Sr. No.	Sample No.	One Ovary				Conception Status
		No. of cells analyzed	Normal for X & 18 (%)	X chr. abn. %	chr. 18 abn. %	
13	FF-013	100	100	0	0	+/missed (abortion)
14	FF-014	100	96	2	2	+
15	FF-015	100	100	0	0	-
16	FF-016	100	90	4	6	-
17	FF-017	100	100	0	0	-
18	FF-018	100	100	0	0	-
19	FF-019	100	97	1	2	-
20	FF-020	100	100	0	0	+
21	FF-021	100	100	0	0	-
22	FF-022	100	98	1	1	-
23	FF-023	100	100	0	0	-
24	FF-024	100	100	0	0	-
25	FF-025	100	100	0	0	+/missed (abortion)
26	FF-026	200	96	3	0	-
27	FF-027	200	99	1	0	+
28	FF-028	200	94	5	1	+
29	FF-029	200	97	0	3	-
30	FF-030	200	95	3	2	-
31	FF-031	200	97	1	2	-
32	FF-032	100	96	3	1	-
33	FF-033	100	96	1	3	-

Sr. No.	Sample No.	One Ovary				Conception Status
		No. of cells analyzed	Normal for X & 18 (%)	X chr. abn. %	chr. 18 abn. %	
34	FF-034	100	100	0	0	+
35	FF-035	100	98	2	0	-
36	FF-036	100	100	0	0	-
37	FF-037	30	28	2	0	-
38	FF-038	100	100	0	0	+
39	FF-039	100	98	1	1	-
40	FF-040	100	100	0	0	-
41	FF-041	100	96	2	2	-
42	FF-042	100	100	0	0	+
43	FF-043	100	100	0	0	-
44	FF-044	100	100	0	0	+/missed (abortion)
45	FF-045	100	98	2	0	+
46	FF-046	100	100	0	0	+
47	FF-047	200	92	6	2	-
48	FF-048	100	97	1	2	+
49	FF-049	200	96	3	1	-
50	FF-050	200	95	3	2	-
51	FF-051	200	99	1	0	-
52	FF-052	100	96	1	3	+
53	FF-053	200	97	2	1	-
54	FF-054	100	100	0	0	-

Sr. No.	Sample No.	One Ovary				Conception Status
		No. of cells analyzed	Normal for X & 18 (%)	X chr. abn. %	chr. 18 abn. %	
55	FF-055	100	100	0	0	-
56	FF-056	200	96	3	1	+
57	FF-057	200	99	1	0	-
58	FF-058	200	94	5	1	-
59	FF-059	200	97	0	3	+
60	FF-060	200	95	3	2	+
61	FF-061	200	97	1	2	-
62	FF-062	200	96	3	1	+
63	FF-063	200	99	1	0	+
64	FF-064	200	94	1	5	-
65	FF-065	200	97	0	3	-
66	FF-066	100	89	10	1	-
67	FF-067	200	95	3	2	-
68	FF-068	200	97	1	2	-
69	FF-069	100	100	0	0	+/missed (abortion)
70	FF-070	100	95	3	2	-
71	FF-071	100	100	0	0	-
72	FF-072	100	99	1	0	-
73	FF-073	100	92	5	2	-
74	FF-074	200	93	5	2	-
75	FF-075	200	95	5	0	-

Sr. No.	Sample No.	One Ovary				Conception Status
		No. of cells analyzed	Normal for X & 18 (%)	X chr. abn. %	chr. 18 abn. %	
76	FF-076	200	95	5	0	-
77	FF-077	200	90	8	2	-
78	FF-078	200	93	5	2	-
79	FF-079	200	93	6	1	-
80	FF-080	100	89	11	0	-
81	FF-081	100	97	3	0	+
82	FF-082	100	98	2	0	+
83	FF-083	100	95	5	0	-
84	FF-084	100	100	0	0	-
85	FF-085	100	93	5	2	+/missed (abortion)
86	FF-086	100	100	0	0	-
87	FF-087	100	100	0	0	+
88	FF-088	100	99	1	0	-
89	FF-089	100	98	2	0	-
90	FF-090	100	100	0	0	-
91	FF-091	100	100	0	0	-
92	FF-092	100	97	3	0	-
93	FF-093	100	98	1	1	+
94	FF-094	100	100	0	0	+
95	FF-095	100	92	6	2	+/missed (abortion)
96	FF-096	100	100	0	0	+

Sr. No.	Sample No.	One Ovary				Conception Status
		No. of cells analyzed	Normal for X & 18 (%)	X chr. abn. %	chr. 18 abn. %	
97	FF-097	100	93	5	2	+/missed (abortion)
98	FF-098	100	99	1	0	-
99	FF-099	100	100	0	0	-
100	FF-100	No Cell				-
101	FF-101	100	99	1	0	+
102	FF-102	No Cell				-
103	FF-103	100	97	3	0	+
104	FF-104	100	100	0	0	-
105	FF-105	No cell				-
106	FF-106	100	100	0	0	+
107	FF-107	100	100	0	0	-
108	FF-108	100	100	0	0	+
109	FF-109	100	92	6	2	-
110	FF-110	100	100	0	0	+
111	FF-111	100	90	9	1	-
112	FF-112	100	100	0	0	-
113	FF-113	100	100	0	0	-
114	FF-114	No cell				+
115	FF-115	300	100	0	0	+
116	FF-116	No cells				-
117	FF-117	200	94	1	5	-

Sr. No.	Sample No.	One Ovary				Conception Status
		No. of cells analyzed	Normal for X & 18 (%)	X chr. abn. %	chr. 18 abn. %	
118	FF-118	200	97	1	2	-
119	FF-119	No cells				-
120	FF-120	100	96	2	2	+
121	FF-121	200	98	1	1	-
122	FF-122	100	100	0	0	-
123	FF-123	100	95	3	2	-
124	FF-124	200	93	3	4	-
125	FF-125	200	96	1	3	-
126	FF-126	100	92	3	5	-
127	FF-127	100	100	0	0	-
128	FF-128	200	87	12	1	-
129	FF-129	200	97	3	0	-
130	FF-130	100	100	0	0	-
131	FF-131	200	98	0	2	-
132	FF-132	200	92	1	7	-
133	FF-133	200	95	2	3	-
134	FF-134	200	96	1	3	+
135	FF-135	200	94	2	4	+
136	FF-136	200	98	2	0	-
137	FF-137	200	98	2	0	-
138	FF-138	200	99	1	0	-

Sr. No.	Sample No.	One Ovary				Conception Status
		No. of cells analyzed	Normal for X & 18 (%)	X chr. abn. %	chr. 18 abn. %	
139	FF-139	200	98	2	0	-
140	FF-140	200	100	0	0	+
141	FF-141	100	93	4	3	+/missed (abortion)
142	FF-142	100	96	4	0	-
143	FF-143	100	98	2	0	+
144	FF-144	200	97	2	1	-
145	FF-145	100	100	0	0	+
146	FF-146	100	92	4	4	-
147	FF-147	200	96	1	3	-
148	FF-148	200	94	2	4	+
149	FF-149	200	98	2	0	+
150	FF-150	200	98	2	0	-
151	FF-151	200	99	1	0	-
152	FF-152	200	98	2	0	-
153	FF-153	100	93	4	3	+
154	FF-154	100	100	0	0	-
155	FF-155	no cell				+
156	FF-156	200	92	6	2	+/missed (abortion)
157	FF-157	100	97	1	2	-
158	FF-158	200	96	3	1	-
159	FF-159	200	95	3	2	-

Sr. No.	Sample No.	One Ovary				Conception Status
		No. of cells analyzed	Normal for X & 18 (%)	X chr. abn. %	chr. 18 abn. %	
160	FF-160	200	99	1	0	-
161	FF-161	100	96	1	3	+
162	FF-162	200	97	2	1	+
163	FF-163	no cell				-
164	FF-164	200	96	3	1	+
165	FF-165	200	95	1	4	+
166	FF-166	200	97	3	0	-
167	FF-167	200	98	2	0	-
168	FF-168	200	94	5	1	-
169	FF-169	200	95	2	3	-
170	FF-170	200	96	3	0	-
171	FF-171	200	99	1	0	-
172	FF-172	200	94	5	1	+/missed (abortion)
173	FF-173	200	97	0	3	-
174	FF-174	200	95	3	2	-
175	FF-175	200	97	1	2	-
176	FF-176	no cell				-
177	FF-177	200	98	1	1	+/missed (abortion)
178	FF-178	100	97	3	0	-
179	FF-179	200	94	2	4	-
180	FF-180	200	98	2	0	+

Sr. No.	Sample No.	One Ovary				Conception Status
		No. of cells analyzed	Normal for X & 18 (%)	X chr. abn. %	chr. 18 abn. %	
181	FF-181	200	98	2	0	-
182	FF-182	200	99	1	0	-
183	FF-183	100	96	2	2	-
184	FF-184	200	97	3	0	+/missed (abortion)
185	FF-185	no cell				+
186	FF-186	200	92	6	2	-
187	FF-187	200	92	1	7	+
188	FF-188	200	95	2	3	+
189	FF-189	200	96	1	3	+/missed (abortion)
190	FF-190	200	94	2	4	-
191	FF-191	200	98	2	0	+/missed (abortion)
192	FF-192	200	98	2	0	-
193	FF-193	200	99	1	0	-
194	FF-194	200	98	2	0	+
195	FF-195	no cell				-
196	FF-196	200	97	3	0	-
197	FF-197	200	95	1	4	-
198	FF-198	100	97	0	3	+
199	FF-199	200	96	1	3	+
200	FF-200	200	99	1	0	+
201	FF-201	200	94	1	5	+

Sr. No.	Sample No.	One Ovary				Conception Status
		No. of cells analyzed	Normal for X & 18 (%)	X chr. abn. %	chr. 18 abn. %	
202	FF-202	200	98	1	1	-
203	FF-203	200	100	0	0	-
204	FF-204	200	98	2	0	-
205	FF-205	100	97	1	2	+/missed (abortion)
206	FF-206	200	94	2	4	-
207	FF-207	100	95	5	0	-
208	FF-208	200	98	2	0	+
209	FF-209	200	98	2	0	-
210	FF-210	200	99	1	0	-
211	FF-211	100	95	3	2	-
212	FF-212	100	97	3	0	-
213	FF-213	100	98	2	0	-
214	FF-214	100	99	1	0	-
215	FF-215	200	92	6	2	-
216	FF-216	100	100	0	0	+
217	FF-217	100	98	1	1	-
218	FF-218	100	97	2	1	+
219	FF-219	100	97	2	1	+
220	FF-220	100	97	2	1	-
221	FF-221	100	100	0	0	+
222	FF-222	100	97	2	1	+

Sr. No.	Sample No.	One Ovary				Conception Status
		No. of cells analyzed	Normal for X & 18 (%)	X chr. abn. %	chr. 18 abn. %	
223	FF-223	100	97	3	0	+
224	FF-224	200	98	2	0	-
225	FF-225	100	94	5	1	-
226	FF-226	100	91	5	4	-
227	FF-227	No cells				-
228	FF-228	100	93	6	1	-
229	FF-229	100	98	2	0	-
230	FF-230	100	89	9	2	+
231	FF-231	no cell				-
232	FF-232	100	96	1	3	+
233	FF-233	100	98	2	0	-
234	FF-234	100	98	2	0	+
235	FF-235	100	97	3	0	-
236	FF-236	100	98	2	0	-
237	FF-237	100	98	2	0	+
238	FF-238	100	100	0	0	-
239	FF-239	100	98	1	1	-
240	FF-240	100	96	3	1	-
241	FF-241	100	98	2	0	+
242	FF-242	100	99	1	0.	-
243	FF-243	100	96	3	1	+

Sr. No.	Sample No.	One Ovary				Conception Status
		No. of cells analyzed	Normal for X & 18 (%)	X chr. abn. %	chr. 18 abn. %	
244	FF-244	200	97	3	0	-
245	FF-245	100	98	2	0	-
246	FF-246	100	94	6	0	+/missed (abortion)
247	FF-247	200	95	3	2	+
248	FF-248	100	100	0	0	+
249	FF-249	100	96	3	1	-
250	FF-250	200	97	3	0	-
251	FF-251	100	98	2	0	+
252	FF-252	200	95	3	2	+
253	FF-253	100	98	2	0	-
254	FF-254	200	97	2	1	-
255	FF-255	100	95	5	0	-
256	FF-256	100	100	0	0	-
257	FF-257	100	96	3	1	-
258	FF-258	200	97	3	0	+/missed (abortion)
259	FF-259	100	98	2	0	+
260	FF-260	200	95	3	2	-
261	FF-261	no cell				-
262	FF-262	100	100	0	0	+
263	FF-263	100	100	0	0	+
264	FF-264	100	100	0	0	-

Sr. No.	Sample No.	One Ovary				Conception Status
		No. of cells analyzed	Normal for X & 18 (%)	X chr. abn. %	chr. 18 abn. %	
265	FF-265	100	100	0	0	-
266	FF-266	100	98	2	0	-
267	FF-267	100	98	1	1	+/missed (abortion)
268	FF-268	100	100	0	0	-
269	FF-269	100	100	0	0	-
270	FF-270	100	98	2	0	-
271	FF-271	200	92	6	2	-
272	FF-272	100	97	2	1	-
273	FF-273	100	100	0	0	+
274	FF-274	100	97	2	1	-
275	FF-275	100	90	8	2	-
276	FF-276	100	97	1	2	-
277	FF-277	100	96	4	0	-
278	FF-278	100	96	2	2	-
279	FF-279	100	97	3	0	-
280	FF-280	100	100	0	0	+
281	FF-281	100	99	0	1	+/missed (abortion)
282	FF-282	100	99	0	1	-
283	FF-283	100	97	2	1	-
284	FF-284	100	95	3	2	+
285	FF-285	100	95	2	3	-

Sr. No.	Sample No.	One Ovary				Conception Status
		No. of cells analyzed	Normal for X & 18 (%)	X chr. abn. %	chr. 18 abn. %	
286	FF-286	100	98	2	0	-
287	FF-287	100	98	2	0	-
288	FF-288	100	95	2	3	-
289	FF-289	100	98	2	0	-
290	FF-290	100	97	3	0	-
291	FF-291	100	100	0	0	-
292	FF-292	100	99	0	1	-
293	FF-293	100	97	2	1	+
294	FF-294	100	100	0	0	+
295	FF-295	100	97	2	1	+
296	FF-296	100	97	3	0	+
297	FF-297	100	93	4	3	-
298	FF-298	100	96	4	0	+
299	FF-299	100	96	3	1	-
300	FF-300	100	92	4	4	-
301	FF-301	200	98	2	0	-
302	FF-302	100	100	0	0	+
303	FF-303	100	100	0	0	-
304	FF-304	100	100	0	0	+
305	FF-305	100	98	2	0	+
306	FF-306	100	92	6	2	-

Sr. No.	Sample No.	One Ovary				Conception Status
		No. of cells analyzed	Normal for X & 18 (%)	X chr. abn. %	chr. 18 abn. %	
307	FF-307	100	98	1	1	+
308	FF-308	100	98	1	1	-
309	FF-309	100	95	4	1	-
310	FF-310	100	98	1	1	-
311	FF-311	100	95	4	1	-
312	FF-312	100	100	0	0	+
313	FF-313	100	98	2	0	-
314	FF-314	100	95	3	2	-
315	FF-315	200	95	3	2	+
316	FF-316	100	100	0	0	-
317	FF-317	100	100	0	0	-
318	FF-318	100	93	5	2	-
319	FF-319	100	99	1	0	+
320	FF-320	100	98	1	1	-
321	FF-321	100	95	4	1	+
322	FF-322	100	100	0	0	+
323	FF-323	100	96	3	1	-
324	FF-324	no cell				-
325	FF-325	100	98	1	1	-
326	FF-326	100	95	4	1	+
327	FF-327	100	100	0	0	+/missed (abortion)

Sr. No.	Sample No.	One Ovary				Conception Status
		No. of cells analyzed	Normal for X & 18 (%)	X chr. abn. %	chr. 18 abn. %	
328	FF-328	100	100	0	0	+
329	FF-329	100	95	2	3	+
330	FF-330	100	97	1	2	-
331	FF-331	100	100	0	0	+
332	FF-332	100	100	0	0	+/missed (abortion)
333	FF-333	100	96	1	3	+
334	FF-334	100	94	3	3	-
335	FF-335	100	100	0	0	-
336	FF-336	100	99	1	0	-
337	FF-337	100	96	2	2	-
338	FF-338	100	96	3	1	+/missed (abortion)
339	FF-339	100	100	0	0	-
340	FF-340	100	100	0	0	-
341	FF-341	100	100	0	0	+
342	FF-342	100	100	0	0	-
343	FF-343	100	95	3	2	-
344	FF-344	100	100	0	0	+
345	FF-345	100	100	0	0	+
346	FF-346	100	98	1	1	-
347	FF-347	100	95	4	1	-
348	FF-348	100	95	3	2	-

Sr. No.	Sample No.	One Ovary				Conception Status
		No. of cells analyzed	Normal for X & 18 (%)	X chr. abn. %	chr. 18 abn. %	
349	FF-349	100	96	2	2	+
350	FF-350	100	96	3	1	+
351	FF-351	100	100	0	0	-
352	FF-352	100	100	0	0	+
353	FF-353	100	100	0	0	+
354	FF-354	100	100	0	0	+
355	FF-355	100	100	0	0	-
356	FF-356	100	94	5	1	-
357	FF-357	100	99	1	0	+
358	FF-358	100	98	1	1	-
359	FF-359	100	99	0	1	-
360	FF-360	100	99	0	1	-
361	FF-361	100	97	2	1	-
362	FF-362	100	95	3	2	-
363	FF-363	100	95	2	3	-
364	FF-364	100	98	2	0	-
365	FF-365	100	98	2	0	-
366	FF-366	no cell				-
367	FF-367	100	98	2	0	-
368	FF-368	no cell				-
369	FF-369	100	100	0	0	-

Sr. No.	Sample No.	One Ovary				Conception Status
		No. of cells analyzed	Normal for X & 18 (%)	X chr. abn. %	chr. 18 abn. %	
370	FF-370	100	99	1	0	-
371	FF-371	100	98	2	0	-
372	FF-372	100	96	4	0	-
373	FF-373	200	88	0	12	-
374	FF-374	100	98	2	0	+
375	FF-375	100	99	1	0	-
376	FF-376	100	93	7	0	+
377	FF-377	100	91	5	4	-
378	FF-378	100	100	0	0	+
379	FF-379	100	95	3	2	-
380	FF-380	100	100	0	0	-
381	FF-381	100	99	1	0	-
382	FF-382	100	97	3	0	-
383	FF-383	100	98	2	0	+
384	FF-384	100	97	1	2	-
385	FF-385	100	95	3	2	-
386	FF-386	100	96	3	1	-
387	FF-387	100	96	1	3	+
388	FF-388	100	100	0	0	+/missed (abortion)
389	FF-389	100	98	2	0	-
390	FF-390	100	95	2	3	+

Sr. No.	Sample No.	One Ovary				Conception Status
		No. of cells analyzed	Normal for X & 18 (%)	X chr. abn. %	chr. 18 abn. %	
391	FF-391	30	28	2	0	+
392	FF-392	100	100	0	0	-
393	FF-393	100	100	0	0	-
394	FF-394	100	100	0	0	-
395	FF-395	100	100	0	0	-
396	FF-396	100	98	2	0	-
397	FF-397	100	98	2	0	-
398	FF-398	100	98	1	1	-
399	FF-399	100	94	4	2	-
400	FF-400	no cell				-
401	FF-401	100	100	0	0	-
402	FF-402	100	97	1	2	+
403	FF-403	100	100	0	0	-
404	FF-404	100	100	0	0	-
405	FF-405	No cells				-
406	FF-406	100	96	0	4	+
407	FF-407	100	100	0	0	-
408	FF-408	100	100	0	0	-
409	FF-409	100	100	0	0	-
410	FF-410	100	98	2	0	+/missed (abortion)
411	FF-411	100	100	0	0	+/missed (abortion)

Sr. No.	Sample No.	One Ovary				Conception Status
		No. of cells analyzed	Normal for X & 18 (%)	X chr. abn. %	chr. 18 abn. %	
412	FF-412	100	98	0	2	-
413	FF-413	100	98	2	0	+
414	FF-414	100	94	6	0	+/missed (abortion)
415	FF-415	100	97	3	0	-
416	FF-416	100	94	2	4	-
417	FF-417	100	95	3	2	-
418	FF-418	100	100	0	0	-
419	FF-419	100	100	0	0	-
420	FF-420	100	98	2	0	-
421	FF-421	100	100	0	0	+
422	FF-422	100	100	0	0	-
423	FF-423	100	98	2	0	-
424	FF-424	100	98	0	2	+
425	FF-425	100	98	2	0	-
426	FF-426	100	100	0	0	-
427	FF-427	100	95	3	2	+/missed (abortion)
428	FF-428	200	94	5	1	-
429	FF-429	200	97	0	3	-
430	FF-430	200	95	3	2	-
431	FF-431	200	97	1	2	-
432	FF-432	100	96	3	1	+

Sr. No.	Sample No.	One Ovary				Conception Status
		No. of cells analyzed	Normal for X & 18 (%)	X chr. abn. %	chr. 18 abn. %	
433	FF-433	100	96	1	3	-
434	FF-434	100	100	0	0	-
435	FF-435	100	100	0	0	+
436	FF-436	100	100	0	0	-
437	FF-437	100	100	0	0	-
438	FF-438	100	94	3	3	-
439	FF-439	100	95	3	2	-
440	FF-440	100	94	2	4	+
441	FF-441	100	100	0	0	-
442	FF-442	100	98	0	2	-
443	FF-443	100	98	2	0	-
444	FF-444	100	100	0	0	-
445	FF-445	100	95	3	2	-
446	FF-446	200	94	5	1	-
447	FF-447	200	95	3	2	+
448	FF-448	200	95	3	2	+
449	FF-449	200	97	1	2	+
450	FF-450	100	96	3	1	-
451	FF-451	100	96	1	3	+
452	FF-452	100	94	2	4	-
453	FF-453	100	100	0	0	-

Sr. No.	Sample No.	One Ovary				Conception Status
		No. of cells analyzed	Normal for X & 18 (%)	X chr. abn. %	chr. 18 abn. %	
454	FF-454	200	97	0	3	+
455	FF-455	200	95	3	2	+
456	FF-456	100	93	4	3	+
457	FF-457	100	100	0	0	+
458	FF-458	100	98	2	0	-
459	FF-459	100	100	0	0	-
460	FF-460	100	95	3	2	-
461	FF-461	200	94	5	1	+
462	FF-462	200	97	0	3	-
463	FF-463	200	95	3	2	-
464	FF-464	200	97	0	3	-
465	FF-465	200	95	3	2	+/missed (abortion)
466	FF-466	100	100	0	0	-
467	FF-467	100	100	0	0	+
468	FF-468	100	98	0	2	-
469	FF-469	100	98	2	0	+
470	FF-470	100	100	0	0	+
471	FF-471	100	95	3	2	-
472	FF-472	100	96	1	3	-
473	FF-473	100	95	3	2	-
474	FF-474	100	98	1	1	-

Sr. No.	Sample No.	One Ovary				Conception Status
		No. of cells analyzed	Normal for X & 18 (%)	X chr. abn. %	chr. 18 abn. %	
475	FF-475	100	94	0	6	-
476	FF-476	100	95	3	2	+
477	FF-477	100	95	3	2	-
478	FF-478	100	98	1	1	+

A select number of cells from the above samples analyzed by FISH are shown in Figures 1-5. Figure 1 shows a photomicrograph of FISH analysis of one of the cells showing normal status for chromosomes 18 and X obtained from follicular fluid in study sample FF – 205. Figure 2 shows a photomicrograph of FISH analysis of one of the cells obtained from follicular fluid showing monosomy 18 in study sample FF – 070. Figure 3 shows a photomicrograph of FISH analysis of one of the cells obtained from follicular fluid showing monosomy X in study sample FF – 097. Figure 4 shows a photomicrograph of FISH analysis of one of the cells obtained from follicular fluid showing trisomy 18 in study sample FF – 200. Figure 5 shows a photomicrograph of FISH analysis of one of the cells obtained from follicular fluid showing trisomy X in study sample FF – 318.

The data presented in Table 1 are consolidated in Table 2:

Table 2

	No. of cases
Number of samples analyzed for either ovary	478
Insufficient cells for analysis	19
Total number of samples analyzed	459
Total number of patients normal for diploid X chromosome	415

after FISH analysis	
Aneuploidy for X chromosome was found in ≥ 5% of cells from isolated follicular fluid	44 (9.58%)

Retrospective analysis of pregnancy or miscarriage status of patients with normal X chromosomes or abnormal X chromosome aneuploidy as identified by FISH is shown below in Table 3:

Table 3

	Patients with X chr. aneuploidy in follicular fluid (44)	Patients with normal X chr. report in follicular fluid (415)
No. of patients conceived	11/44 (25%)	148/415 (35.66%)
No. of patients miscarried	7/11 (63.63%)	21/148 (14.19%)

Out of 478 follicular fluid samples processed from either ovary, sufficient cells for analysis were not obtained in 19 cases. The results of FISH on follicular fluid cells in the remaining 459 cases are shown in table 1. In our laboratory, 1-4% aneuploid cells are considered to be within the normal range. This has been found in other laboratories as well (Vysis AneuVysion® Multicolor DNA Probe Kit (Part Number 32-161075 & 33-161075 & 35-161075) package insert, Table 4, attached hereto as Appendix A, and incorporated herein by reference). Of the samples analyzed by FISH, 415 patients showed a normal X chromosome pattern, while 44 patients showed a mosaic follicular fluid pattern (≥5% aneuploidy of the X chromosome). For the individuals displaying a mosaic aneuploidy for the X chromosome, karyotyping and FISH analysis were also performed on blood samples. Eighteen of these 44 (40.9%) patients had low-grade mosaicism (≥5% aneuploidy) of the X chromosome in blood as well, confirming the follicular fluid results. The remaining 26 patients were normal for X chromosome aneuploidy from blood

analysis, suggesting that mosaicism in these 26 patients may be limited to the gonads (gonadal mosaicism).

Upon further analysis we noticed that 11 out of 44 patients (25%) with aneuploidy detected by FISH were pregnant. On the other hand, 148 of the 415 patients (35.66%) having normal follicular fluid FISH results were pregnant. Hence, the pregnancy rate in patients with aneuploidy detected in follicular fluid cells was less than that for patients with normal follicular fluid FISH reports. This suggests that gonadal mosaicism correlates with reduced fertility rates among women using *in vitro* fertilization to conceive. Moreover, of the 11 patients with X chromosome aneuploidy who conceived, 7 patients miscarried (63.63%). Conversely, of the 148 patients with normal follicular fluid FISH results who had conceived, only 21 patients miscarried (14.19%), which correlates with the normal miscarriage rate in the general population. This suggests that gonadal mosaicism for the X chromosome aneuploidy correlates with an increased risk of miscarriage.

Example 2

Identification of gonadal mosaicism using cells from follicular fluid from either ovary by FISH for abnormalities of chromosomes 13 and 21.

Cells and slides were prepared as described in Example 1. However, the follicular fluid samples were obtained from patients with a history of children affected by trisomy 13 or 21. The patients, themselves, had also been diagnosed, through blood analysis, as having a mosaic pattern of aneuploidy for chromosomes 13 or 21. Probes specific for chromosome 13 (LSI 13 Spectrum Green) and 21 (LSI 21 Spectrum Orange) were used (AneuVysion EC DNA probe kit, Vysis). The results of these analyses are shown below in Table 4:

Table 4

Sr. No.	Sample No.	Identification of abnormalities for chromosome 13 and 21 with cells obtained from follicular fluid from either ovary	Conception Status

		No. of cells analyzed	Normal for 13 & 21 (%)	chr. 13 abn. %	chr. 21abn. %	
1	FF-A01	200	92	8	0	+
2	FF-A02	200	94	6	0	-
3	FF-A03	200	99	0	1	-
4	FF-A04	200	91	1	8	+
5	FF-A05	200	88	2	10	-
6	FF-A06	200	96	2	2	-
7	FF-A07	200	97	1	2	-
8	FF-A08	200	92	3	5	-
9	FF-A09	200	99	1	0	-
10	FF-A10	200	93	3	4	+

A select number of cells from the above samples analyzed by FISH are shown in Figures 6-8. Figure 6 shows a photomicrograph of FISH analysis of one of the cells obtained from follicular fluid showing normal status for chromosomes 13 and 21 in study sample FF – A01. Figure 7 shows a photomicrograph of FISH analysis of one of the cells obtained from follicular fluid showing trisomy 13 in study sample FF – A03. Figure 8 shows a photomicrograph of FISH anaslysis of one of the cells obtained from follicular fluid showing trisomy 21 in study sample FF – A05.

Example 3

Identification of gonadal mosaicism using cells from follicular fluid obtained from both ovaries of an individual by FISH for abnormalities of chromosomes 18, X, and Y.

This experiment was conducted in the same manner as Example 1, except the cell sample of follicular fluid was obtained from both the ovaries instead of either of ovary as in Example 1. The results of these analyses are shown below in Figure 5:

Table 5

Sr. No.	Sample No.	Left				Right				Conception status
		No. of cells analyzed	Normal for X & 18 (%)	X chr. abn. (%)	Chr. 18 abn. (%)	No. of cells analyzed	Normal for X & 18 (%)	X chr. Abn. (%)	chr. 18 abn (%)	
1	FF-479	100	92	6	2	100	86	8	2	-
2	FF-480	100	92	6	2	100	91	5	4	-
3	FF-481	100	100	0	0	100	100	0	0	-
4	FF-482	100	97	3	0	100	100	0	0	-
5	FF-483	100	100	0	0	100	100	0	0	-
6	FF-484	100	100	0	0	100	98	2	0	+
7	FF-485	100	98	2	0	100	100	0	0	-
8	FF-486	100	95	4	0	100	98	0	2	-
9	FF-487	100	94	3	3	100	98	2	0	-
10	FF-488	100	94	5	1	100	93	4	3	-
11	FF-489	100	94	0	6	100	97	0	3	+
12	FF-490	100	99	0	1	100	94	6	0	+
13	FF-491	50	96	2	2	100	97	3	0	-
14	FF-492	100	82	14	4	100	92	6	2	+/missed abortion
15	FF-493	100	97	0	3	100	95	3	2	-
16	FF-494	100	100	0	0	100	98	2	0	-

Sr. No.	Sample No.	Left				Right				Conception status
		No. of cells analyzed	Normal for X & 18 (%)	X chr. abn. (%)	Chr. 18 abn. (%)	No. of cells analyzed	Normal for X & 18 (%)	X chr. Abn. (%)	chr. 18 abn (%)	
17	FF-495	100	100	0	0	100	91	5	4	-
18	FF-496	100	95	5	0	100	95	5	0	-
19	FF-497	100	96	1	4	100	96	2	2	+
20	FF-498	100	96	4	0	100	98	1	1	-
21	FF-499	100	100	0	0	100	94	4	2	-
22	FF-500	100	94	3	3	100	93	2	5	+
23	FF-501	100	95	3	2	100	97	0	3	-
24	FF-502	100	97	0	3	100	97	0	3	-
25	FF-503	100	100	0	0	100	97	3	1	+
26	FF-504	100	95	2	3	100	93	3	4	-
27	FF-505	100	96	4	0	100	97	0	3	+
28	FF-506	100	92	2	6	100	95	1	4	-
29	FF-507	100	95	3	2	100	94	2	4	-
30	FF-508	100	100	0	0	100	94	4	2	-
31	FF-509	100	97	0	3	100	94	4	2	+
32	FF-510	100	96	2	3	100	95	1	4	-
33	FF-511	100	96	2	2	100	98	2	0	+
34	FF-512	100	99	1	0	100	100	0	0	-
35	FF-513	100	94	5	2	100	95	5	0	-
36	FF-514	100	96	0	4	100	93	3	4	+ / missed abortion

Sr. No.	Sample No.	Left				Right				Conception status
		No. of cells analyzed	Normal for X & 18 (%)	X chr. abn. (%)	Chr. 18 abn. (%)	No. of cells analyzed	Normal for X & 18 (%)	X chr. Abn. (%)	chr. 18 abn (%)	
37	FF-515	100	97	3	0	100	98	0	2	+
38	FF-516	100	97	1	2	100	96	2	2	-
39	FF-517	100	97	1	2	100	98	2	0	-
40	FF-518	100	98	0	2	100	97	3	0	-
41	FF-519	100	97	2	1	100	96	4	2	-
42	FF-520	100	93	2	5	100	99	0	1	-
43	FF-521	100	90	5	5	100	94	5	2	+
44	FF-522	100	98	1	1	100	97	2	1	-
45	FF-523	100	95	3	2	100	95	2	3	-
46	FF-524	100	91	3	6	100	93	5	2	+ / missed abortion
47	FF-525	100	95	3	2	100	93	2	5	-
48	FF-526	100	100	0	0	100	94	2	4	-
49	FF-527	100	97	2	1	100	98	1	1	+ /missed abortion
50	FF-528	100	95	2	3	100	86	7	7	-
51	FF-529	100	100	0	0	100	100	0	0	-
52	FF-530	100	93	2	3	100	100	0	0	-
53	FF-531	100	96	3	1	100	94	2	5	+/ missed abortion
54	FF-532	100	97	2	1	100	94	2	4	+

Sr. No.	Sample No.	Left				Right				Conception status
		No. of cells analyzed	Normal for X & 18 (%)	X chr. abn. (%)	Chr. 18 abn. (%)	No. of cells analyzed	Normal for X & 18 (%)	X chr. Abn. (%)	chr. 18 abn (%)	
55	FF-533	100	100	0	0	100	100	0	0	-
56	FF-534	100	95	3	2	100	93	3	4	-
57	FF-535	100	100	0	0	100	100	0	0	-
58	FF-536	100	93	5	2	100	95	5	0	-
59	FF-537	100	93	1	5	100	90	3	7	-
60	FF-538	100	97	1	2	100	92	2	6	+
61	FF-539	100	98	0	2	100	94	0	6	-
62	FF-540	100	98	0	2	100	94	4	2	-
63	FF-541	100	96	2	2	100	95	1	4	-
64	FF-542	100	96	2	2	100	96	2	2	-
65	FF-543	100	87	6	7	100	92	3	5	-
66	FF-544	100	93	3	4	100	94	2	4	+
67	FF-545	100	95	3	2	100	91	5	4	+ / abortion
68	FF-546	100	94	2	4	100	93	3	4	+
69	FF-547	100	97	0	3	100	96	2	2	-
70	FF-548	100	97	1	2	100	100	0	0	+
71	FF-549	100	93	4	3	100	96	2	2	-
72	FF-550	100	92	3	5	100	91	4	5	-
73	FF-551	100	95	1	4	100	97	2	3	-
74	FF-552	100	89	2	9	100	93	2	5	-

Sr. No.	Sample No.	Left				Right				Conception status
		No. of cells analyzed	Normal for X & 18 (%)	X chr. abn. (%)	Chr. 18 abn. (%)	No. of cells analyzed	Normal for X & 18 (%)	X chr. Abn. (%)	chr. 18 abn (%)	
75	FF-553	100	96	2	2	100	94	2	5	-
76	FF-554	100	96	1	3	100	98	1	1	-
77	FF-555	100	96	0	4	100	95	1	4	-
78	FF-556	100	91	4	5	100	94	0	6	+
79	FF-557	100	93	3	4	100	95	2	3	-
80	FF-558	100	94	2	4	100	94	2	4	-
81	FF-559	100	86	6	8	100	83	8	9	-

[0001] The data in Table 5 were consolidated and analyzed and the results are shown below in Table 6:

Table 6

	No. of cases
Number of samples analyzed for Both ovaries	81
Insufficient cells for analysis	0
Total number of samples analyzed	81
Total number of patients normal for X chromosomal abnormality on FISH analysis	67
Aneuploidy for X chromosome $\geq$ 5% in follicular fluid in either ovary	06
Aneuploidy for X chromosome $\geq$ 5% in follicular fluid in both ovaries	08

Retrospective analysis of pregnancy or miscarriage status in normal patients compared to patients with aneuploidy for chromosome X, as determined by FISH analysis, is shown below in Table 7:

Table 7

	Patients with X chr. aneuploidy in follicular fluid in both ovary	Patients with X chr. aneuploidy in follicular fluid in either ovary	Patients with normal X chr. report in follicular fluid
No. of patients conceived	2/8 (25%)	2/6 (33.33%)	16/67 (23.88%)
No. of patients miscarried	2/2 (100%)	1/2 (50%)	3/16 (18.75%)

Of the 81 follicular fluid samples processed, 67 samples did not display X chromosome abnormality (≤ 5% aneuploid cells; Table 6). For 6 of the remaining 14 cases, aneuploidy only was found in samples from one ovary, while in the other 8 cases, aneuploidy was detected in follicular fluid samples from both ovaries (Table 6).

In retrospective analysis, 2 of the 8 patients having X chromosome aneuploidy in both ovaries conceived but both miscarried. Two of the 6 patients having X chromosome aneuploidy in either of the two ovaries analyzed conceived, one of which miscarried. In comparison, of the 67 patients normal for the X chromosome, 16 conceived (23.88%), of which, only 3 miscarried (18.75%).

Example 4

Identification of gonadal mosaicism using cells from follicular fluid for karyotype analysis (Coverslip Method).

1) Collection of cells from follicular fluid.

Cells from follicular fluid were collected from 4 patients undergoing *in vitro* fertilization treatment as described in Example 1.

2) Cell culture

The follicular fluid sample from each patient was centrifuged at 1000 rpm for 10 minutes to pellet the cells. The supernatant was discarded leaving behind approximately 4 mL of

fluid above the pellet, and the pellet was then resuspended in the remaining fluid. Approximately 0.5 mL was pipetted onto a sterile coverslip placed in a Petri dish. The sample was allowed to dry for several minute before adding 0.5 mL of culture medium (Amniomax; Sigma) and incubating overnight at a temperature of 37°C. and 5% CO_2. The following day, approximately 2 mL of culture medium was added and the plates were incubated for an additional two days. The cultures were monitored daily under a microscope for fibroblast formation and additional culture medium was added as necessary. When the cultures were confluent, the supernatant was removed and fresh medium was added. Cells were harvested two days later by adding 20 µL of Demicolcine (Sigma) to the culture, incubating in a CO_2 incubator for 20 minutes, and adding 2 mL of a hypotonic solution (0.75M KCl and 0.6% sodium citrate). The cultures were then incubated for 20 minutes at 37°C.

3) Cell fixation and karyotyping

Five drops of fresh fixative at room temperature were slowly added to the cultures, and the cultures were incubated at 37°C. for 10 minutes. Another 1 mL aliquot of fixative was added, and another 2 mL were added 10 minutes later. After 20 minutes at room temperature, all of the fixative was removed and 2 mL of fresh fixative was added and again incubated for 20 minutes at room temperature. This last fixation step was repeated twice. The coverslip was removed from the petridish and kept on damp tissue for drying. The coverslip was then mounted on a clean, dry, slide using DPX mounting medium. The slide was stained following the GTG banding method (The AGT Cytogenetics Laboratory Manual, 3rd Edition, 1997, P 259-324) and air-dried. The slide was then observed under oil immersion to check for sharp banding. A minimum of 20 to 30 metaphases were analyzed for numerical and structural abnormalities.

4) Results

Of the 20 metaphases analyzed in a sample from one individual, monosomy X was detected in 3 metaphases (Figure 9). This result was also confirmed in 1 of 20 metaphases analyzed in a blood sample.

Example 5

Identification of gonadal mosaicism using cells from follicular fluid by karyotype analysis (Culture flask method).

1) Follicular fluid cell collection

Follicular fluid samples were collected as described in Example 1 from six individuals undergoing *in vitro* fertilization procedures.

2) Cell culture and fixation

The follicular fluid sample from each individual was centrifuged at 1000 rpm for 10 minutes to allow the cells to pellet. Supernatant was discarded, leaving behind approximately 4 mL of fluid above the pellet. The pellet was then resuspended in the remaining supernatant. 2 mL of the cell suspension was added to two tissue culture flasks respectively. Culture medium (Amniomax, Sigma) was added to the flasks, and the cultures were incubated at 37°C. for 6-8 days. An additional 2 mL of culture medium was added to each flask, and the flasks were incubated for another two days. The cultures were observed everyday for fibroblast formation under a microscope and supplemented with culture medium as necessary. When fibroblast colonies were found in the cultures, the cells were harvested by adding 50 µl of Demecolcine (Sigma) and incubating at about 37°C. for two to three hours. The cells were next transferred to a centrifuge tube. EDTA solution was added to the flask, and the flask was tapped and the contents were again transferred to the centrifuge tube. This was followed by a wash of trypsin solution to the flask for two to three minutes, and again, transferred to the centrifuge tube.

About 2 mL of hypotonic solution (0.75M KCl and 0.6% sodium citrate) was added to the centrifuge tube containing the cultured cells and the tube was kept at about 37°C. for about 20 minutes. To this 10 to 12 drops of fresh, room temperature fixative was slowly added and the tube was kept at about 37°C. for about 10 minutes. The tube was then centrifuged at 1000 rpm for 10 minutes. The supernatant was discarded, leaving behind about 0.5 mL of solution above the pellet. The pellet was resuspended in the residual supernatant. Initially, 1 mL of fresh chilled fixative was added; to this about another 5 mL of fixative was added and the suspension was left at 2 to 8°C. for 16-20 hours. The fixed samples were centrifuged and the supernatant discarded leaving behind about 0.5 mL solution above the pellet. About 6 mL of fresh cold (2 to 8°C.) Carnoy's fixative was added, the contents were centrifuged and more fixative was added. This fixation step was repeated twice.

3) Karyotyping

Several drops of the cell suspension were placed on a chilled wet slide. The slide was then heated over a water bath set at 60° to 68°C. for 2 seconds and then transferred to a hot plate set at 45°C. for one minute. The slide was observed under a phase contrast microscope at 10X magnification for metaphases with long and well-spread chromosomes. The slide was stained by GTG banding method as described above and air-dried. The slide was then observed under oil immersion to check for good and sharp banding. A minimum of 20 metaphases were analyzed for numerical and structural abnormalities.

4) Results

Of the 30 metaphases analyzed in a sample from a patient with the history of offspring with Down's syndrome, trisomy 21 was detected in 4 metaphases (Figure 10). In contrast, all metaphases observed through blood sample analysis were normal. These results suggest the presence of gonadal mosaicism in this individual.

As shown in Examples 1 to 5, gonadal mosaicism, including low-grade gonadal mosaicism, can be identified in cells obtained from follicular fluid. This indicates that cells obtained from follicular fluid can serve as an effective means to identify chromosomal abnormalities in a population of symptomatic and asymptomatic individuals.

WE CLAIM:

1. A method of identifying a reproductive system abnormality comprising:

   a) obtaining cells from follicular fluid;

   b) subjecting the cells to genetic analysis;

   wherein the identification of at least one chromosomal abnormality in a portion of the cells is indicative of a reproductive system abnormality.

2. The method of claim 1, wherein the follicular fluid is obtained from a human.

3. The method of claim 2, wherein the cells are somatic cells.

4. The method of claim 2, wherein the follicular fluid is obtained during an *in vitro* fertilization procedure.

5. The method of claim 2, wherein the follicular fluid is obtained during an intracytoplasmic sperm injection procedure.

6. The method of claim 1, wherein the follicular fluid is obtained from one ovary of a subject.

7. The method of claim 1, wherein the follicular fluid is obtained from both ovaries of a subject.

8. The method of claim 1, wherein the genetic analysis is fluorescent *in situ* hybridization.

9. The method of claim 1, wherein the genetic analysis is karyotyping.

10. The method of claim 1, wherein the genetic analysis is DNA sequencing.

11. The method of claim 1, wherein the genetic analysis is a method selected from the group consisting of comparative genome hybridization (CGH), multicolor-banding (MCB), quantitative FISH (Q-FISH), polymerase chain reaction (PCR), genetic bit analysis (GBA), multiplex sequencing, SNaPshot,

MassEXTEND, MassArray, microarray ligation, microarray miniseq, tag arrays, arrayed primer extention (APEX), microarray primer extension, GOOD assay, coded microspheres, restriction fragment length polymorphism analysis (RFLP), allele specific oligonucleotide (ASO) analysis, methylation-specific PCR (MSPCR), pyrosequencing analysis, acycloprime analysis, reverse dot blot, GeneChip microarrays, dynamic allele-specific hybridization (DASH), peptide nucleic acid (PNA) and locked nucleic acids (LNA) probes, TaqMan, Molecular Beacons, intercalating dye, FRET primers, AlphaScreen, SNPstream, Invader assay, Template-directed incorporation (TDI), fluorescence polarization, sequence-coded oligonucleotide ligation assays, ligase chain reaction, padlock probes, rolling circle amplification, and colorimetric oligonucleotide ligation assay (OLA).

12. The method of claim 11, wherein the comparative genome hybridization is performed with metaphase chromosomes or a CGH-array.

13. The method of claim 1, wherein the chromosome abnormality is gonadal mosaicism.

14. The method of claim 13, wherein the gonadal mosaicism is low-grade gonadal mosaicism.

15. The method of claim 1, wherein the chromosome abnormality is selected from the group consisting of aneuploidy, translocation, deletion, microdeletion, inversion, and duplication.

16. The method of claim 15, wherein the aneuploidy is complete or partial trisomy.

17. The method of claim 16, wherein the trisomy is trisomy 13, trisomy 16, trisomy 18, trisomy 21, trisomy 22, XXY, XYY, or XXX.

18. The method of claim 15, wherein the aneuploidy is complete or partial monosomy.

19. The method of claim 18, wherein the monosomy is monosomy X, monosomy 13, monosomy 16, monosomy 18, monosomy 21, or monosomy 22.

20. The method of claim 15, wherein the aneuploidy is complete or partial nullisomy.

21. The method of claim 20, wherein the nullisomy is for chromosome 13, 16, 18, 21, 22, X, or Y.

22. A method for assaying an increased risk of infertility in an animal comprising:

a) obtaining cells from follicular fluid of the animal; and

b) subjecting the cells to genetic analysis,

wherein the identification of at least one chromosomal abnormality in a portion of the cells is indicative of an increased risk of infertility.

23. The method of claim 22, wherein the animal is a human.

24. The method of claim 23, wherein the follicular fluid is obtained during an *in vitro* fertilization procedure or an intracytoplasmic sperm injection procedure.

25. The method of claim 24, wherein an embryo resulting from the *in vitro* fertilization procedure undergoes pre-implantation genetic analysis.

26. The method of claim 25, wherein the genetic analysis demonstrates that the embryo does not have a chromosome abnormality, and the embryo is transferred into the animal's uterus.

27. A method of identifying gonadal mosaicism in a subject comprising:

a) obtaining cells from follicular fluid isolated from the subject;

b) subjecting the cells to genetic analysis;

wherein a portion of the cells have at least one chromosomal abnormality indicating that the subject has gonadal mosaicism.

28. The method of claim 27, wherein the subject is human.

29. The method of claim 28, wherein the follicular fluid is obtained during an *in vitro* fertilization procedure or an intracytoplasmic sperm injection procedure.

30. The method of claim 27, wherein the gonadal mosaicism is low-grade gonadal mosaicism.

31. The process according to claims hereinabove substantially as herein described with reference to the examples and figures.

FIGURE 1

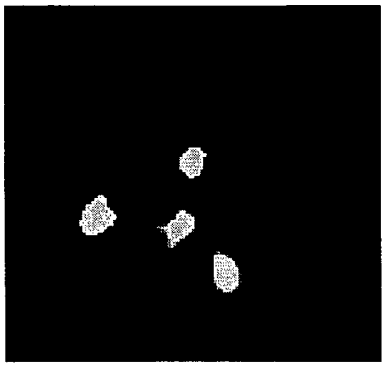

FIGURE 2

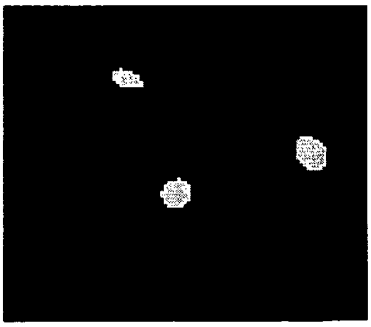

FIGURE 3

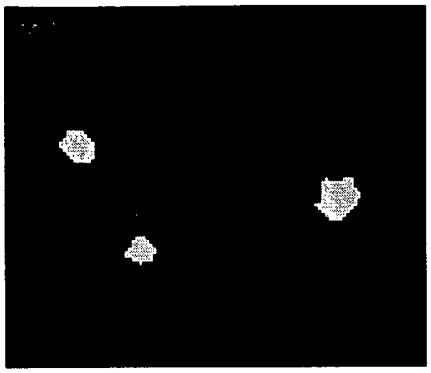

FIGURE 4

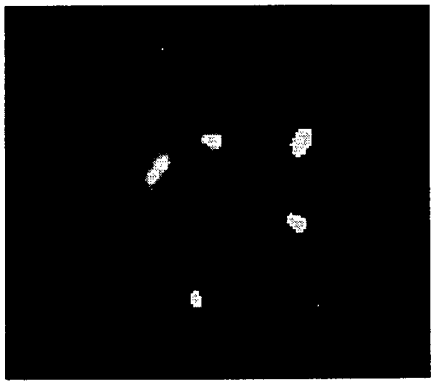

FIGURE 5

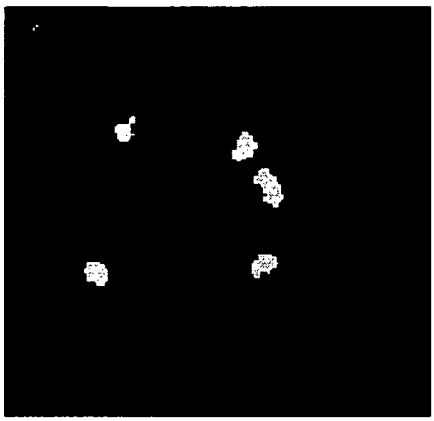

FIGURE 6

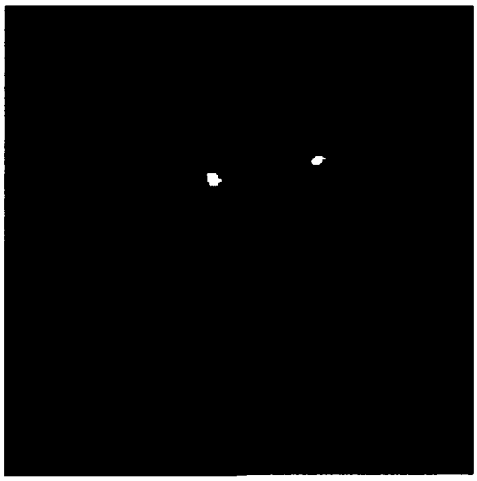

FIGURE 7

FIGURE 8

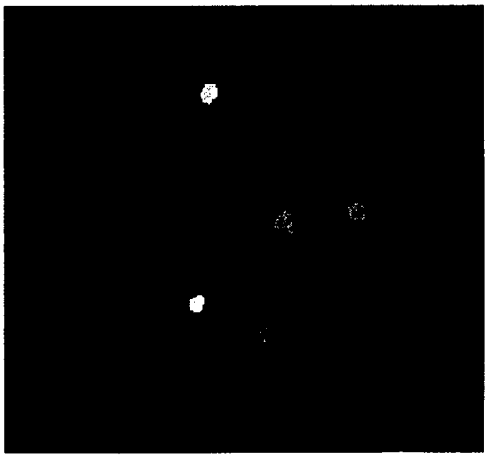

FIGURE 9

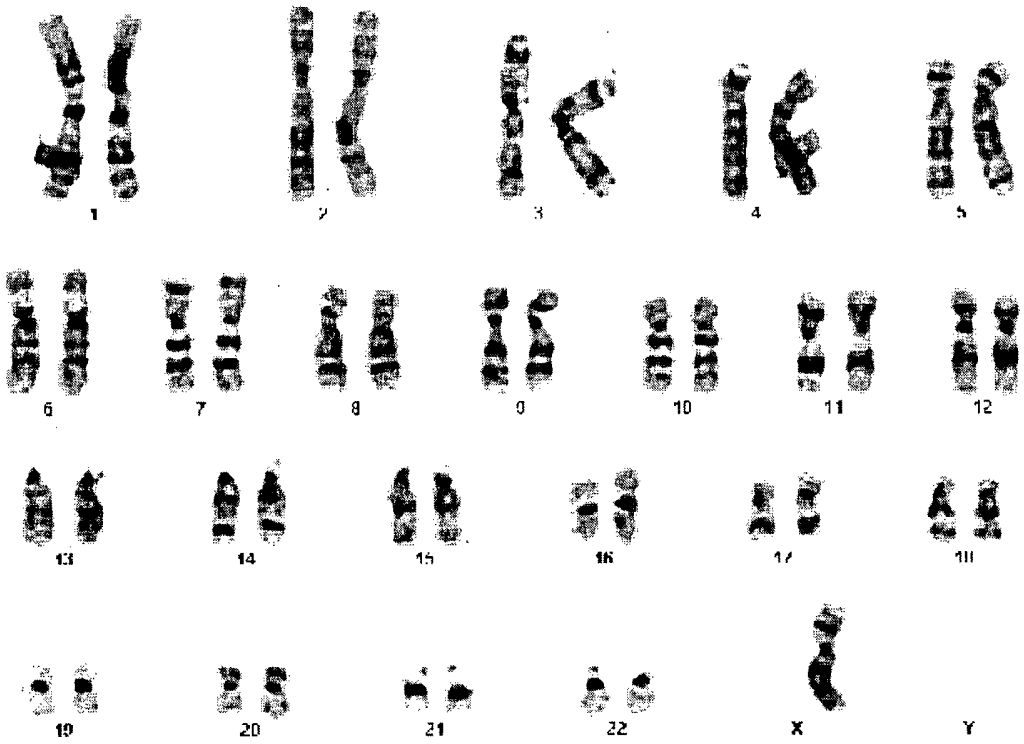

FIGURE 10