

Bridge Submissions to ESHRE 2008

Gabriel A, Giddings L, Griffin D, Handyside A, Thornhill A
PCR On A Slide: A Novel Platform To Analyse Single Cells For Preimplantation Genetic Diagnosis.

[Accepted for Oral \(O-165; i67\)](#)

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Nuclear organisation in totipotent human nuclei and its relationship to chromosomal abnormality.

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3D ultrasound and Antimullerian hormone determination as predictors of ovarian response to controlled stimulation in assisted reproduction.

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Increased levels of chromosomal aneuploidy in sperm from men requiring ICSI lead to increased embryo aneuploidy.

[Accepted for Poster \(P517; i207\)](#)

Handyside AH, Chatzimeletiou K, Grigorova M, Doubravska S, Robertson L, Finch K and Thornhill AR

An excess of monosomies in aneuploid embryos from women of advanced maternal age.

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Griffin D, Fonseka K, Finch K, Abogrein A, Handyside A, Thornhill A, Hickson N,

Nuclear organisation in human sperm: Preliminary evidence for altered sex chromosome centromere position in infertile males

[Not Accepted](#)

Balet R, Bellinge R, Thornhill A, Handyside A, Iammarrone E, Shaw L, Grudzinskas G

Day surgery sperm retrieval using PESA or TESE with elective freezing: A retrospective review of 6 years experience and 292 cases.

[Not Accepted](#)

Iammarrone E, Gillott DJ, Tse S, Grudzinskas JG, Ojha K

Role of the three-dimensional ultrasound in the evaluation of the uterine cavity in women undergoing Assisted Reproductive Technologies.

[Not accepted](#)

Role of the three-dimensional ultrasound in the evaluation of the uterine cavity in women undergoing Assisted Reproductive Technologies.

Iammarrone E, Gillott DJ, Tse S, Grudzinskas JG, Ojha K

Introduction

The endometrial cavity is routinely assessed by 2D Ultrasound or by invasive procedures such as Aquascan or Hysteroscopy. The availability of 3D Ultrasound (3DUS), a new imaging technique, enabled us to assess the uterine cavity with high resolution. The aim of our study was to evaluate the uterine cavity with 3D US and to assess if there is a correlation with implantation in women undergoing Assisted Reproductive Technologies.

Materials and methods

78 women underwent 3D US (GE Voluson 730) of the uterine cavity prior to treatment. Correlation between the uterine cavity morphology and positive pregnancy rate has been examined.

Results

A regular uterine cavity was observed in 61 women (group A) and 17 women showed an irregular uterine cavity (group B). In group A there were 10 women with intramural and/or sub-serosal fibroids. The irregularities observed in Group B were: 4 arcuate uterus, 7 polyps, 5 sub-mucosal fibroids and 1 anechogenic area. There was no difference between group A and B in terms of age (37.1 ± 4.3 vs 37.2 ± 4.8). The number of oocytes collected was similar between the two groups (8.4 ± 4.0 vs 8.6 ± 5.6) as were the number of embryos derived (5.0 ± 2.9 vs 4.6 ± 3.5) and the number of embryos transferred (1.9 ± 0.3 vs 1.7 ± 0.4). The positive pregnancy rate was comparable between the two groups (34.4% in group A and 29.4% in group B ($p > 0.05$)). Of the 10 women in group A with intramural and/or sub-serosal fibroids we observed 40% positive pregnancy rate.

Conclusions

Pregnancy rates among women with a normal uterine cavity, but with the presence of fibroids were slightly higher than in women with an irregular cavity. I think numbers too small for this group to say much at all. Otherwise you could argue that having fibroids was actually a positive prognostic indicator compared with normal uterus without fibroids (based on these data?) Though this is not statistically significant it does indicate that the presence of intramural or sub-serosal fibroids do not negatively impact on pregnancy rates. However, this is a small group of patients and a larger study is required for adequate statistical analysis. We believe that 3D US is the best available non-invasive technique for assessment of the uterine cavity.

PCR On A Slide: A Novel Platform To Analyse Single Cells For Preimplantation Genetic Diagnosis

Gabriel A*, Giddings L, Griffin D, Handyside A, Thornhill A

Introduction

Single cell PCR for preimplantation genetic diagnosis (PGD) requires high efficiency and accuracy. Allele dropout (ADO), the random amplification failure of one of the two parental alleles, can result in serious misdiagnosis for compound heterozygous or autosomal dominant conditions. Strategies to combat ADO include the use of efficient lysis buffers, optimised PCR protocols for amplification of small DNA fragments, increased denaturation temperatures in the initial cycles of amplification, fluorescent PCR to increase sensitivity and the inclusion of multiple linked markers flanking the gene defect. Less attention has been focussed on PCR kinetics and the particular thermal cycling platform on which each single cell reaction is performed. Here we investigate the use of a slide-based thermal cycler system for amplifying

single cells in single microlitre volumes to facilitate rapid loading of single cells, reducing the volume of reagents required and potentially improving PCR efficiency by optimizing temperature cycling and increasing sensitivity.

Materials and Methods:

Single buccal cells were isolated from an individual heterozygous for a tetranucleotide short tandem repeat (STR) on chromosome 13 (D13S251), washed and placed individually into water droplets (<1 µl) distributed across a grid of 48 circular hydrophilic areas ('wells') on an otherwise hydrophobic glass slide designed to allow PCR amplification in situ without mixing between samples as an alternative to the conventional approach using microcentrifuge tubes (AmpliGrid™, Advantix, Germany). Wells 1-36 contained single cells, 37-40 5-cell samples, 41-45 negative controls and 46-50 gDNA positive controls (Sigma-Aldrich control DNA). After the samples were air dried and lysed in 0.75µl lysis buffer (Advantix), 0.25 µl PCR master mix was added to give a final volume of 1µl and overlaid with 5µl sealing solution (Advantix).

Following initial denaturation (95°C, 3 min), thermal cycling took place on a dedicated ultra-compact, high-speed slide cycler (AmpliSpeed™, Advantix) with 30 cycles of denaturation (94°C, 30s); annealing (53°C, 45s) and extension (72°C, 30s) followed by a final extension step (72°C, 5 min). All Cy5 labelled PCR products were detected using a DNA analyzer (CEQ 8000, Beckman Coulter).

Results:

PCR amplification of the STR, D13S251, using the slide based system and dedicated ultra rapid thermal cycler, was achieved in control and single cell samples following 30 cycles in 1.5µl reaction volumes taking only 1h 18min, which is within the time-frame required for clinical PGD application and comparable to conventional fast-ramping tube-based thermal cyclers. Amplification efficiency for single buccal cells was high (31/36 (86%) and ADO was only detected in 1/31 samples (3%).

Conclusions:

A slide-based system combined with a dedicated ultra-rapid thermal cycler has a number of advantages for amplification from single cells for PGD and other applications. These include: (i) reduced reaction times (ii) and significantly reduced reagent volumes and cost per sample (iii) high amplification efficiency and low ADO rates (iv) simpler cell loading and sample preparation facilitating higher throughput.

Nuclear organisation in human sperm: Preliminary evidence for altered sex chromosome centromere position in infertile males

Griffin D, Fonseka K, Finch K, Abogrein A, Handyside A, Thornhill A, Hickson N

Introduction:

Chromatin packaging is involved extensively throughout spermatogenesis; thus abnormalities associated with it (e.g. chromosome rearrangements), lead to male infertility. Translocations, inversions, aneuploidy and increased sperm disomy are all chromosomal correlates of compromised semen parameters. The objective of this study was to test the hypothesis of whether there is another correlate, i.e. altered chromosome position in the sperm head. Chromosome position is a well-established marker of nuclear organisation and alterations in established patterns can lead to disease phenotypes such as cancer, laminopathies and epilepsy. Normal mammalian sperm adopts a pattern characteristic of nuclear organisation with the centromeres aligning towards the nuclear centre alongside the post-meiotic sex chromatin. Specifically, we wished to determine the likelihood of centromeres from specific chromosomes to locate towards the centre of the sperm head nucleus.

Material and Methods:

Sperm samples were collected from men participating in sperm donation or those currently under treatment for infertility at The London Bridge Fertility, Gynaecology and Genetics Centre, at the National Infertility Centre, Abumeliana Fertility Clinic, Farah fertility centre or Misurata Infertility Centre in Libya. Group I consisted of 10 sperm donors with normal semen parameters according to WHO Guidelines (1999). Group II was composed of 15 men currently undergoing IVF treatment with reduced semen parameters ranging from Oligozoospermic to severe OATs diagnoses. Fresh ejaculate was re-suspended in Tris/NaCl buffer, spread on to a glass slide and dehydrated in an ethanol series. Decondensation in DTT was followed by denaturation, overnight hybridisation and formamide post-hybridisation washes. Probes used were the Vysis Multivision panel (13, 16, 18, 21, 22) and Aneuvision panel (X, Y, 18) in 10 normozoospermic males and 15 with various compromised semen parameters. Probe signal positions were then recorded using a template overlay consisting of five rings of linearly increasing volumes. The frequency with which specific probes localised to different rings was compared using Chi Square tests.

Results:

We examined 7240 signals from 8 locus-specific probes for 7 different chromosomes. Results were consistent with a "chromo-centre" pattern for the chromosomes analysed (with the exception of loci on chromosomes 13 and 21) in Group I. In Group II, signals for X, Y and 18 centromeres were more likely to be distributed in a random pattern but this was not consistent for all infertility phenotypes.

Conclusions:

One obvious practical implication of our results is that probes found to occupy domains at the nuclear centre (such as the sex chromosomes and the centromeric probes) are more likely to be scored as monosomies as a result of signal overlap. For this reason, the reliability of centromeric probes for aneuploidy screening warrants further investigation. Our results also support the utility of screening infertile men for chromosome position in sperm heads.

Nuclear organisation in totipotent human nuclei and its relationship to chromosomal abnormality

[Finch K](#), [Fonseka KGL](#), [Ioannou D](#), [Hickson N](#), [Barclay Z](#), [Mantzouratou A](#), [Delhanty J](#), [Chatzimeletiou K](#), [Thornhill A](#), [Handyside A](#), [Griffin D](#)

Introduction:

Studies of nuclear organisation, most commonly determining the nuclear location of chromosome territories and individual loci, have furthered our understanding of nuclear function, differentiation and disease and there is increasing interest in the significance of regions of non-randomly organized chromatin within the nucleus (the so-called chromo-centers). By examining loci on different chromosomes we tested the hypotheses that:

1. Totipotent human blastomeres adopt a nuclear organisation akin to that of committed cells;
2. Nuclear organisation is different in chromosomally abnormal blastomeres;
3. Human blastomeres adopt a "chromo-center" pattern.

Analysis of IVF embryos allows valuable insight into the cell biology of totipotent human nuclei. Our objective was to compare chromosome positioning in nuclei of totipotent human blastomeres and committed cells using extrapolations from images created during clinical preimplantation genetic screening (PGS) for chromosomal aneuploidy in human embryos.

Material and Methods:

Study group I comprised over 200 karyotypically normal lymphocyte nuclei from a fertile man (committed cells); Study group II comprised 218 human embryonic (blastomere) nuclei showing no detectable abnormality (which we estimate have a 60-80% chance of being chromosomally normal); Study group III comprised 333 chromosomally aneuploid human embryonic nuclei; Study group IV comprised 75 nuclei derived from chromosomally "chaotic"

embryos. A total of 6892 FISH signals were scored in blastomere nuclei. All blastomere images were obtained following PGS cycles undertaken at The London Bridge Fertility, Gynaecology and Genetics Centre and University College London Centre for Preimplantation Genetic Diagnosis. The probes used were commercially available (Vysis, Abbott laboratories) hybridising to chromosomes 13, 15, 16, 18, 21, 22, X and Y. Probe signal positions were recorded using a template overlay consisting of five rings of linearly increasing volumes. The frequency with which specific probes localised to different rings was compared using Chi Square tests.

Results:

Group I (lymphocyte) nuclei demonstrated a non-random pattern for all autosomal nuclei as expected. In contrast, Group II illustrated a pattern more consistent with random organisation. Comparisons between Groups II and III (blastomere) nuclei suggest that Group III nuclei displayed a significant non-random pattern for all autosomal loci but not the sex chromosome loci. Group IV showed tendencies toward a random distribution as with group II. Interestingly, centromeric loci on chromosomes 15 and 16 were noted as peripheral in group I but central in Group III providing some evidence of a chromo-center arrangement.

Conclusions:

One practical implication of our results is that probes found to occupy domains at the nuclear centre (such as the sex chromosomes and the centromeric probes) are more likely to be scored as monosomies as a result of signal overlap. For this reason, the reliability of centromeric probes for aneuploidy screening warrants further investigation. Our results may provide clues to the nature of totipotency in human cells and have implications for preimplantation diagnosis and nuclear transfer.

Day surgery sperm retrieval using PESA or TESE with elective freezing: A retrospective review of 6 years experience and 292 cases.

Balet R, Bellinge R, Thornhill A, Handyside A, Iammarrone E, Shaw L, Grudzinskas JG

Introduction

IVF techniques can utilize both fresh and frozen sperm for oocyte insemination. Frozen sperm are most frequently used with male factor infertility, when sperm is obtained by procedures such as percutaneous epididymal sperm aspiration (PESA), or testicular sperm extraction (TESE). We wished to review our experience of surgical sperm retrieval with elective sperm freezing with a particular focus on percutaneous sperm retrieval and assess successful sperm retrieval, utilisation of samples, cycle and pregnancy outcomes as well as patient post-operative experience.

Material and methods

All day surgery PESA and multiple percutaneous-multiple biopsy (TESE) sperm retrieval cases with elective freezing were reviewed for a 6-year period. All cases were performed at a large private infertility centre. Azoospermic patients were referred to a single clinician who performed all the cases (RB) in the day surgery unit under local anaesthetic with or without intravenous sedation. Patients with obstructive azoospermia (OA) were treated with Percutaneous Epididymal Sperm Aspiration (PESA). If no sperm was retrieved or in cases of non-obstructive azoospermia (NOA), Testicular Sperm Extraction (TESE) was performed and an average of 10 to 15 samples were taken from each testis with a 14 G Cook Core biopsy needle. Patients were discharged from the clinic within 2 hours following the procedure. A post-operative questionnaire was given to each patient after the procedure and responses recorded and analysed.

Results

274 patients underwent 292 procedures (6 had 2 attempts and 2 had 3 attempts). 129 cases were NOA, 99 had OA (55 failed vasectomy with or without reversal, 14 bilateral congenital absence of vas deferens, 22 of other obstructive origin) and 2 had anejaculation. Sperm was retrieved in 97 out of 99 cases of obstructive azoospermia (98%) and in 25 out of 64 cases of

non-obstructive azoospermia (39%). Sperm was found in 4 cases when previous open biopsies were negative. 143 cycles of ICSI started led to 140 (97.9%) oocyte collections and 130 (90.9%) embryo transfer procedures. Clinical pregnancy rates per transfer and implantation rates were 32.3 % and 19.7 % respectively. To date, 44.7 % couples have had a clinical pregnancy with five miscarriages and one ectopic pregnancy. 34 babies have been born so far with no major abnormality observed. 7 pregnancies are still ongoing. TESE sperm was used in 53 cycles and PESA sperm in 85 cycles. Pregnancy rates per transfer or per couple were significantly different depending on the origin of the sperm (PESA 36.47 % and 53.44 % versus TESE 20.75% and 28.9%).

Thirteen patients with successful sperm retrieval are awaiting IVF and 10 patients have not contacted the clinic even though sperm has been available for at least 6 months. 46/274 (17%) post-operative forms were received and 6 (13%) patients experienced severe pain, 12 (26%) complained of swelling and bruising but all patients were able to resume normal activity within 5 days. Only one patient experienced a haematoma, which required no further surgical treatment.

Conclusions

Percutaneous sperm retrieval is safe, simple, well accepted by patients, and gives acceptable results in terms of sperm yield. Elective sperm freezing simplifies patient management and avoids unnecessary ovarian stimulation.

3D ultrasound and Antimullerian hormone determination as predictors of ovarian response to controlled stimulation in assisted reproduction.

Tse S, Gillott DJ, Iammarrone E, Grudzinskas JG, Ojha K

Introduction

We have examined the relationship between circulating anti-mullerian hormone (AMH) and antral follicle count in patients prior to fertility treatment. We wish to establish the extent to which 3D ultrasonography is predictive of ovarian response to stimulation. It is anticipated that this approach may significantly improve the clinical management of patients undergoing controlled ovarian stimulation (COS).

Materials & Methods

Patients (n = 32) attending for baseline examinations prior to infertility treatment underwent 3D ultrasound scan for antral follicle count and ovarian volume between days 1-7 of their menstrual cycle (GE Voluson 740 ultrasound machine). AMH and follicle stimulating hormone (FSH) were also determined at baseline scan. The number of eggs collected following standard COS was recorded. Patients with polycystic ovarian syndrome (PCOS) were excluded.

Results

A strong correlation between total antral follicle count and circulating AMH was observed ($R^2 = 0.7522$). Mean circulating AMH concentrations were significantly lower in patients with less than 10 antral follicles (n = 15; AMH 4.9 pM) when compared to patients with more than 10 antral follicles (n = 18; AMH 24.3 pM; p = 0.00114). A strong correlation between AMH and the number of eggs collected was apparent (n = 8; $R^2 = 0.8244$), however, no correlation between FSH and the number of eggs collected was found ($R^2 = 0.0139$). The mean number of eggs collected in patients with AMH levels less than 10pM was 3.2 compared to a mean number of eggs of 18.7 in patients who had AMH levels above 10pM (p = 0.00013). Total ovarian volume showed a slight positive correlation with total antral follicle count ($R^2 = 0.3480$) and AMH levels ($R^2 = 0.2240$). No correlation between total ovarian volume and number of eggs collected was observed ($R^2 = 0.0977$). There was no correlation between AMH and FSH levels ($R^2 = 0.057$). The mean age of the patients was 39.7 (range 32 – 47). There was a trend towards lower AMH values with increasing age, but this was not significant ($R^2 = 0.1098$). Similarly, there was a general negative trend in the number of eggs collected with increasing age ($R^2 = 0.1780$).

Conclusions

These preliminary data indicate a strong correlation between circulating AMH and antral follicle count. Both these values appear to be far better predictors of the response to COS than FSH determination and age considerations. Despite relatively low numbers of patients that have had egg collection so far, patients with AMH above 10pM or total antral follicle counts higher than 10 had significantly higher numbers of oocytes collected. In addition, AMH does not exhibit the cyclic variation that is seen with FSH making determinations more reliable. Ovarian volume did not correlate well with number of eggs collected, though it did exhibit a slight correlation with circulating AMH levels. We believe that antral follicle count as determined by 3D ultrasound along with circulating AMH determinations provide an improved method of managing patients embarking on COS in assisted reproductive techniques, both in terms of obtaining the optimal number of oocytes and avoiding complications such as ovarian hyperstimulation syndrome.

Increased levels of chromosomal aneuploidy in sperm from men requiring ICSI lead to increased embryo aneuploidy.

Thornhill A, Abogrein A, Finch K, Chatzimeletiou K, Handyside A, Grudzinskas JG, Griffin DK

Introduction

Intracytoplasmic sperm injection (ICSI) offers the best chance for many couples suffering from severe male infertility to conceive; however potential drawbacks of ICSI should be addressed closely in terms of the risk from the paternal gamete of chromosomes aneuploidies in resulting embryos. Many couples experience failure of embryo implantation after ICSI and 30% of pregnancies result in fetal wastage due to aneuploidy. Approximately 60% of spontaneous abortions and 5% of stillbirths are caused by chromosomal aberrations in natural conception however it still not clear whether this risk is increased as a result of ICSI compared to the general population. The role of increased chromosomal aneuploidy in the sperm of men requiring ICSI owing to reduced semen parameters and in preimplantation embryos is well documented. The extent to which increased aneuploidy in sperm from men requiring ICSI leads to increased levels of aneuploidy in the resultant ICSI-derived embryo is less clear. Our objective was to investigate possible paternal effects involved in the transmission of aneuploidy through ICSI.

Material and Methods:

Sperm samples from men with a range of spermatogenic impairment conditions ranging from oligozoospermia to severe oligoasthenoteratozoospermia and corresponding embryos not transferred following the treatment by ICSI were collected and analysed for chromosomal abnormalities using fluorescence in situ hybridization (FISH) with probes for seven chromosomes (13, 16, 18, 21, 22, X and Y (Vysis, Abbott Laboratories) in two sequential layers.

Results

Males were split into three groups of low (0.7% - 2.1 %), medium (2.2% - 4.4%), and high (5.0% - 13.1%) sperm aneuploidy for these seven chromosomes (maternal ages were near identical in each group) and then assayed to ask whether the resultant embryos showed a significant increase in chromosome abnormality. The numbers of abnormal embryonic cells were 58.0%, 63.5%, and 73.4% in the low, medium and high groups respectively (a small but statistically significant increase in each case) indicating that increased sperm aneuploidy does indeed contribute to the overall levels of chromosome abnormality in the resultant embryo despite the overwhelming maternal contribution to embryo abnormalities. Results for individual chromosomes also showed similar increases.

Conclusions

These data provide some of the first direct indications that ICSI using sperm from men with aberrant levels of sperm aneuploidy increases the risk of chromosomally abnormal embryos.

An excess of monosomies in aneuploid embryos from women of advanced maternal age

Handyside AH, Chatzimeletiou K, Grigorova M, Doubravska S, Robertson L, Finch K, Thornhill AR

Introduction

Preimplantation genetic screening (PGS) for chromosomal aneuploidy by cleavage stage biopsy and sequential multicolour fluorescence in situ hybridisation (FISH) has been criticised because of the limited accuracy of single cell analysis resulting from chromosomal mosaicism and technical errors. In particular, an excess of monosomies has been reported when multiple FISH probes are used for analysis of single interphase nuclei suggesting that, for example, overlapping signals may be a common cause of misdiagnosis. For these reasons, we routinely offer follow up analysis of all the remaining nuclei of biopsied embryos identified as aneuploid, both to confirm the screening result and to provide information on the frequency of uniform aneusomies (presumed to be of meiotic origin) versus chromosomal mosaicism. Here we report that for uniform aneusomies in women of advanced maternal age, instead of the expected 1:1 ratio of trisomies to monosomies, there is a genuine and significant excess of monosomies for the chromosomes analysed, though there is variation between chromosome pairs.

Materials and methods

Following PGS for advanced maternal age (AMA), with patient consent, zona intact biopsied embryos identified as aneuploid or not selected for transfer or cryopreservation, were spread on day 4, 5 or 6 following egg retrieval using either 0.1 or 0.3% Tween in 0.01 N HCl. Sequential multicolour FISH for chromosomes 22, 21, 18, 16 and 13 (Multivision, Abbott Diagnostics) and X, Y and 18 (Aneuvysion, Abbott Diagnostics) was then used for follow up analysis. Embryos were identified as uniformly aneuploid if a large majority of nuclei (>85%) had the identical aneusomy or combination of aneusomies and there was no evidence of postzygotic non-disjunction or other identifiable abnormal pattern of chromosome segregation. The incidence of uniform trisomies and monosomies was then compared with a two sided Sign test.

Results

In 64 PGS cycles for AMA, follow up analysis was successful in 197 embryos of which 97 (49%) were identified as uniformly aneusomic for one or more of the 6 chromosome pairs analysed (see table). Among these embryos 63 (65%) had single, 28 (29%) double and 6 (6%) triple aneusomies. Aneusomy for chromosome 22 was the most prevalent (36%) while aneusomies for the sex chromosomes were relatively rare. For 5 out of 6 chromosome pairs, there was an excess of monosomies compared with trisomies and although not statistically significant for individual chromosomes, the overall excess of monosomies was highly significant ($p=0.004$). In contrast, almost equal numbers of trisomies and monosomies were identified for chromosome 21.

	Chromosome						Total
	22	21	18	16	13	XY	
Trisomy	20	9	5	9	6	0	49
Monosomy	27	10	12	19	9	4	81
Total (%)	47 (36)	19 (15)	17 (13)	28 (21.5)	15 (11.5)	4 (3)	130
T/M ratio	0.74	0.9	0.42	0.47	0.67	0.00	0.6
p (2 sided)	0.25	0.99	0.15	0.1	0.61	0.25	0.004

Conclusions

Errors in maternal meiosis, underlying the age related increase in aneuploid oocytes, and, consequently uniform aneuploidy following fertilisation, are thought to be caused either by non-disjunction in the first or second meiotic divisions or premature predivision of chromatids. With both of these mechanisms, it has been assumed that the distribution of the non-disjoined chromosomes or single chromatids, is random resulting in equal numbers of nullosomic or disomic oocytes. As maternal meiosis involves unequal cell divisions resulting in the extrusion of the two small polar bodies from the much larger oocyte, one explanation for the excess of monosomies in embryos is that these abnormally segregating chromosomes tend to be retained in the polar bodies. Further studies using molecular genetic markers will be needed to confirm the meiotic origin of these aneuploidies and their parental origin, and potentially, differentiate between the different mechanisms causing these errors.