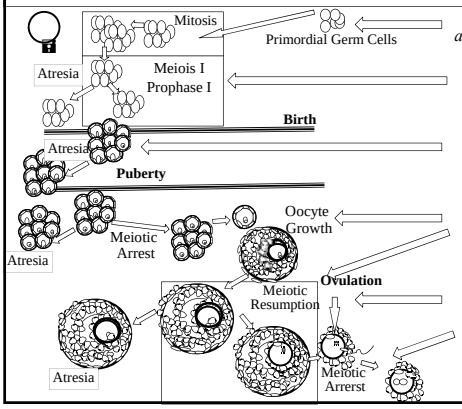
ESHRE CAMPUS:
7th Workshop on Mammalian Folliculogenesis and Oogenesis,
Stresa, Italy – 19-21 April 2012

At which stages throughout oogenesis can disturbances occur that predispose to meiotic errors?

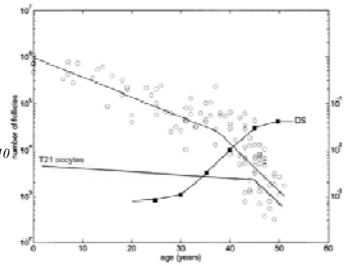
Ursula Eichenlaub-Ritter
University of Bielefeld
Gene Technology/Microbiology
Bielefeld
Germany

Stages at which errors in chromosome segregation are initiated



Constitutional trisomy in primary oocytes: Mosaicism of trisomy 21

Hulten et al. Curr Genomics. 2010 Sep;11(6):409-19.



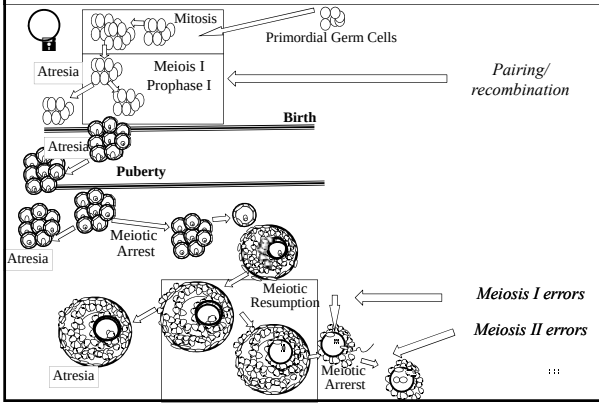
Increased proportion of T21 oocytes in the ageing ovary. The OMI hypothesis proposes that the T21 oocytes lag behind during development, resulting in higher proportions of the total oocyte pool over time. The figure illustrates the predicted number of T21 oocytes from birth until menopause (pink line) in comparison to the total (black circles) based on follicle counts (left hand Y axis) by Faddy [142]. The observed incidence (right hand Y axis) of 121 DS births (black squares) is represented by the data of Morris et al. [143]. The offset of the (pink) line showing the predicted number of T21 oocytes is based on the 0.56% mosaicism observed by Hultén et al. [24]. The slope is an approximation generating the expected DS birth rates with increasing maternal age. Note that the figure illustrates the principle of this hypothesis only and the lines drawn are based on rather uncertain estimates. (Reproduced from [25])

Constitutional trisomy in primary oocytes: Mosaicism of trisomy 21

No evidence for segregation patterns expected from trisomic primary oocytes (Handyside et al. Eur J Hum Genet (1012) 1-6)

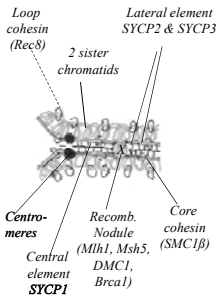
Select group of patients may have gonadal mosaicism predisposing to trisomy 21 (e.g. Conn et al., J Med Genet 1999, 36).

Stages at which errors in chromosome segregation are initiated



Pairing and recombination

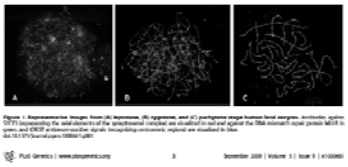
Synaptonemal complex



Disturbed recombination may contribute to recurrent spontaneous abortions (e.g. patients carrying certain alleles of SYCP genes).

(Bolor et al., Am J Hum Genet. 2009 Jan;84(1):14-20.; Stoffs et al. Reprod Biomed Online. 2011 Jan;22(1):65-71; Mitzutani et al. Hum Reprod. 2011 May;26(5):1259-66.)

Pairing and recombination



Genetic linkage and analysis of MLH1 foci in spread fetal human oocytes suggest that there are 3500 to 4500 exchanges/oocyte (Chen et al., PLOS Genet 2009; 5(9) e10000661)

Table 6. Summary of data correlating recombination defects with the genesis of maternally derived trisomies.

Trisomy	Achiasmate bivalents	Distal exchanges	Proximal exchanges	Comments (references)
13	yes	no	no	Estimated 25–33% of cases associated with achiasmate bivalents (2, 3)
16	no	yes	no	No known correlation of achiasmate bivalents, but distally located exchanges reported for more cases (5, T Hassold and H Hall, unpublished observations)
18	unknown	unknown	unknown	"Rare" if strong, no available information on origin
21	yes	no	no	Estimated 10% of cases associated with achiasmate bivalents (6)
21	yes	yes	yes	Estimated 40% of cases involve achiasmate bivalents; distally located exchanges important contributor to meiosis I cases and proximal exchanges important for apparent reduced X axis (7, 8)
22	yes	no	no	Estimated 25% of cases associated with achiasmate bivalents (9)

Maternal trisomies involve frequently achiasmatic chromosomes

Pairing and recombination

Sexual dimorphism:
Knockout of genes in recombination:
Early and irreversible meiotic arrest in spermatocytes
Oocytes progress to advanced stages- mainly first meiotic errors.
(reviewed by Cohen & Morelli, Reproduction 2005, 130, 761)

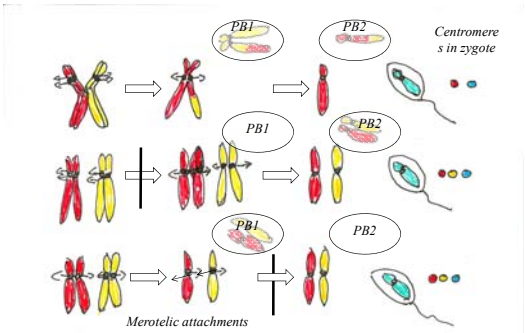
Oocytes tolerate univalents:

Mlh1$^{-/-}$ oocytes have reduced recombination and increased univalents but progress to anaphase I suggesting that:

1.stringency of the SAC to detect chromosomes attached to one pole is low in oocytes

2.or univalents can satisfy the SAC by forming bipolar attachments (Nagaoka et al..Curr Biol. 2011 Apr 26;21(8):651-7).

Pairing and recombination



Recognized as MI error with tracing centromeric polymorphisms/
Recognized as MII error in arrays based on hybridisation to probe

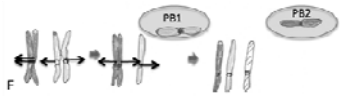
Pairing and recombination

MI and MII normal

Absence of recombination or precocious loss of cohesion between sister chromatid arms and centromeres increases susceptibility to first and also second meiotic errors!

MII nondisjunction due to predivion at MI involving both homologs

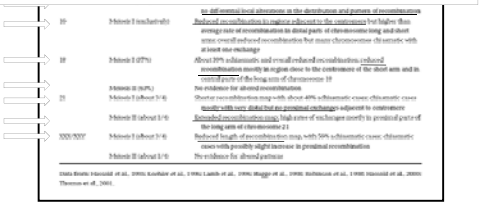
MI predivision leading to chromatid in 1PB and errors at MII



Pairing and recombination

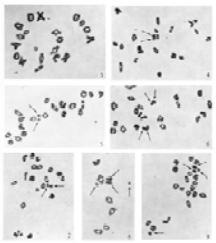
Are disturbances in recombination at the basis of maternal <u>age-related</u> aneuploidy?

Altered recombination appears involved in some specific trisomies!



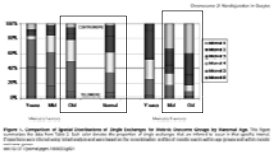
Suggestion that failure in recombination predestines ‚late' oocytes to errors in segregation at advancd age (production line)

Chiasma Frequency and Maternal Age in Mammals

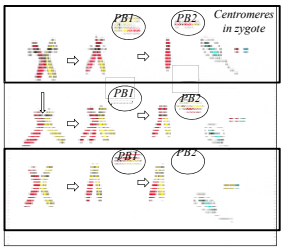


Are there differences in recombination according to age and the predictions by the production line hypothesis?

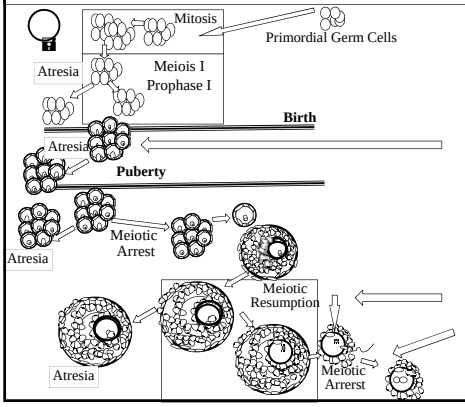
Extra chromosomes in trisomies have different recombinational history and correlation to maternal age



Oliver et al., 2010, Plos Genet. 4
Oliver et al., 2011, Hum Genet Dec 2011



Stages at which errors in chromosome segregation are initiated



Damage during long arrest

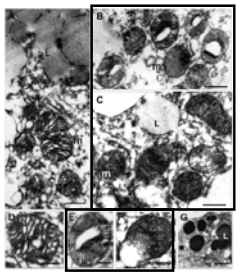
Meiosis I errors

Meiosis II errors

:::

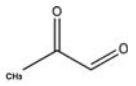
Damage during long arrest

Mitochondria in granulosa and oocytes from aged human ovaries are frequently morphologically (and possibly functionally) aberrant and aberration may cause a vicious cycle in ROS-induced damage



Tatone et al., 2006, Mol. Hum. Reprod. 11, 655-660

Age-associated damage to mitochondria and membranes by ‚advanced glycation end products' (AGEs)



Methylglyoxal: highly reactive metabolite from glycolysis leads to formation of ‚advanced glycation end products' (AGEs) and ROS

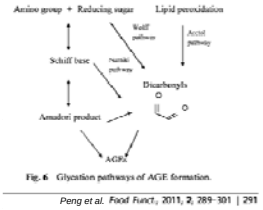
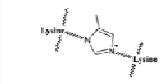


Fig. 6 Glycation pathways of AGE formation.

Peng et al. Food Funct., 2011, 2, 289–301 | 291



Methylglyoxal-lysine dimer (MOLD)

Can we mimick age-related changes in oocytes? Exposure to methylglyoxal during maturation

Human Reproduction, Vol.26, No.7 pp. 1843–1855, 2011
Advanced Access publication on May 9, 2011 doi:10.1093/humrep/der140

human reproduction ORIGINAL ARTICLE Reproductive biology

Evidence that carbonyl stress by methylglyoxal exposure induces DNA damage and spindle aberrations, affects mitochondrial integrity in mammalian oocytes and contributes to oocyte ageing

Carla Tatone[1], Tanja Heizenrieder[2], Giovanna Di Emidio[1], Patrick Treffon[2], Fernanda Amicarelli[3], Thorsten Seidel[4], and Ursula Eichenlaub-Ritter[2,*]

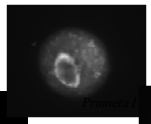
[1]Department of Health Sciences, University of L'Aquila, Via Vetoio, 67100 L'Aquila, Italy [2]Faculty of Biology, Gene Technology Microbiology, University of Bielefeld, 33501 Bielefeld, Germany [3]Department of Basic and Applied Biology, University of L'Aquila, Via Vetoio, 67100 L'Aquila, Italy [4]Faculty of Biology, Dynamic Cell Imaging, University of Bielefeld, 33501 Bielefeld, Germany

*Correspondence address. Tel: +49-(0)-521-106-4402/4907; Fax: +49-(0)521-106-6015; E-mail: ue@uni-bielefeld.de

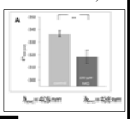
Submitted on December 14, 2010; resubmitted on March 7, 2011; accepted on March 10, 2011

Dynamic distribution of mitochondria in mouse oocytes

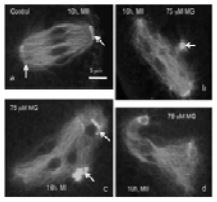
JC-1: Low redox potential: green High redox potential: red



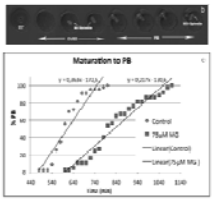
Stage-specific burst in ATP levels in association with dispersal/accummulation (Eichenlaub-Ritter et al., Mitochondrion 2010)



Exposure to methylglyoxal induces spindle aberrations and meiotic delay



No significant increase in aneuploidy in young oocytes



Can we improve follicular environment and prevent age-related disturbances?
How can lifestyle factors influence susceptibility?

Caloric restriction in mice reduced maternal age-related mitochondrial clustering and aneuploidy in MII oocytes (Salesniemi et al., PNAS, 108(30) 2011)

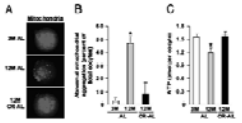


Fig. 4. CR maintains normal mitochondrial dynamics in oocytes of aged females (A) Representative mitochondrial distribution in MII oocytes from 3M AL-fed, 12M AL-fed, and 12M CR-AL-fed mice (MitoTracker staining shown in red) (B) Incidence of abnormal mitochondrial aggregations in MII oocytes from 3M AL-fed, 12M AL-fed, and 12M CR-AL-fed mice (mean ± SEM, $n = 35$–40 oocytes analyzed per group from three independent experiments using 6–11 mice per group; *$P < 0.05$ versus 3M AL-fed to mean). (C) Cytoplasmic ATP levels in individual MII oocytes from 3M AL-fed, 12M AL-fed, and 12M CR-AL-fed mice (mean ± SEM, $n = 38$–105 total oocytes analyzed per group from five to seven independent experiments using a total of 5–21 mice per group; *$P < 0.05$ versus 3M AL-fed females).

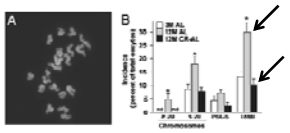


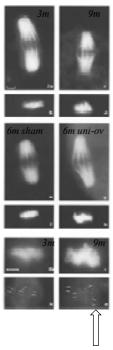
Fig. 2. Aging-associated aneuploidy in MII oocytes is prevented by CR. (A) Example of a typical chromosome spread of an MII oocyte containing 20 chromosomes (DAPI staining of DNA shown in white). (B) Incidence of hyperploidy, hypoploidy, and PSCS (and total chromosomal defects from all these endpoints combined) in MII oocytes of 3M AL-fed, 12M AL-fed, and 12M CR-AL-fed females (mean ± SEM, $n = 18$–28 mature oocytes analyzed per group in each experiment replicated four times using a total of 20–34 mice per group; *$P < 0.05$ versus 3M AL-fed females; nd, none detected).

Hormonal environment and/or pool size can be one factor predisposing to nondisjunction.

1.High FSH may affect spindle formation (Roberts et al., Biol Reprod 2005; 72: 107–118) and is associated with trisomic conceptions (not low AMH in this study; Kline et al., Hum Reprod 2011; 26: 1537-1550).

1.Reduced pool size/ low AMH was associated with a trisomic conceptus (Haadsma et al., Hum Reprod 2010; 25, 552-528) in another study.

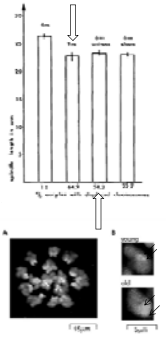
Unilateral ovariectomy and analysis of spindles and chromosome behaviour in mouse MII oocytes



(Eichenlaub-Ritter, Chandley and Gosden, 1988):

Spindle length is age –dependent; Chromosome displacement appears dependent on pool size.

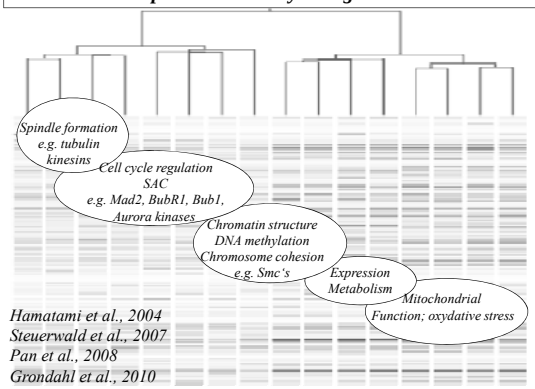
Centromeres of sister chromatids appear more separated in aged compared to young oocytes! (Merriman et al., Biol Reprod 2011)



Loss of cohesion with ageing!

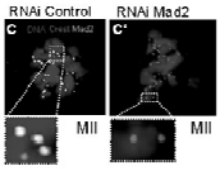
Merriman et al., Biol Reprod 2011

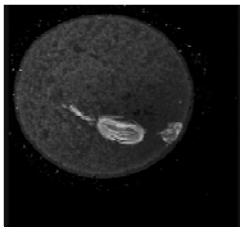
Consistently ageing affects transcript levels of gene products in spindle and cell cycle regulation



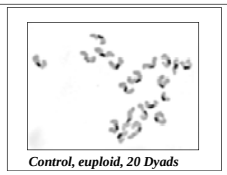
Hamatami et al., 2004
Steuerwald et al., 2007
Pan et al., 2008
Grondahl et al., 2010

Young oocytes express SAC: Knockdown of the SAC predisposes to unchecked progression to anaphase I in presence of defective spindles

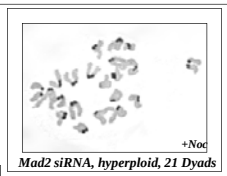




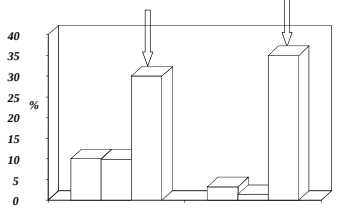
Knockdown of the SAC predisposes to aneuploidy



Control, euploid, 20 Dyads



Mad2 siRNA, hyperploid, 21 Dyads



- Control, unspec. RNAi, n = 90
- Mad2 siRNA, n = 131
- Noc., Mad2 siRNA, n = 20

SAC prevents aneuploidy upon additional disturbance!

Resolution of erronemous attachment (merotelic) of microtubules appears particularly important in oogenesis with acentriolar spindles

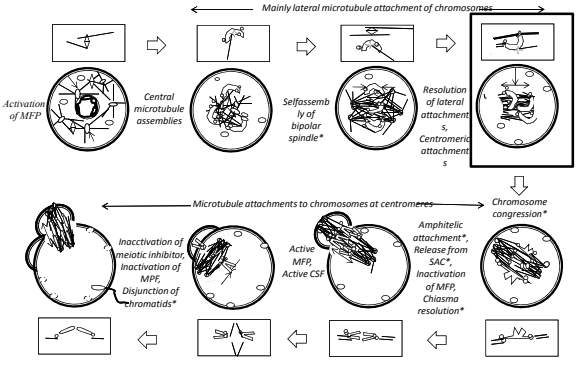
Differentially expressed transcripts involved in SAC and chromosome segregation

Transcript	Relative Expression
SAC	
Bub1	1.28
Bub1b	0.64
Cdc20	0.81
Cdc27	1.21
Chek1	0.67
Cnb1	0.76
Congression/Kinetochore MT	
AurkB	1.47
Kif2c (MCAK)	0.54
Plk1	0.51
Ndc	0.56
Cenpe	0.70
Spindle Assembly	
Kifc1 (HSET)	0.55
Numa1	0.67
Ran	1.44
Tpx2	1.17
Dynein light chains	1.50

MCAK (mitosis centromere associated kinesin)depolymerase is implicated in resolving improper microtubule attachments.

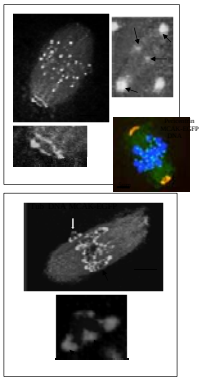
Pan et al., 2008

Acentriolar spindle formation in oocytes is associated with long prometaphase I with repetitive merotelic attachment and reorientation of sister chromatids and homologous chromosomes

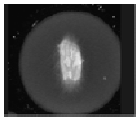


Modified from Kitajima et al., Cell 2011)

Disturbances in expression of components like MCAK that help resolve improper attachments causes congression failure/delay



Subtle alterations in expression may contribute to meiotic error and reduced developmental capacity



Knockdown induced arrest and congression failure;

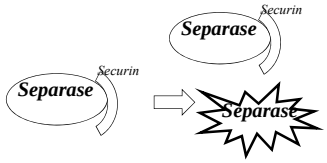
double knockdown with Mad2: increased hypoploidy

Vogt et al., Mol Hum Reprod, 2010

Further evidence that altered expression may contribute to age-associated reduced developmental potential of the human oocyte

Gene expression profiles of single human mature oocytes in relation to age

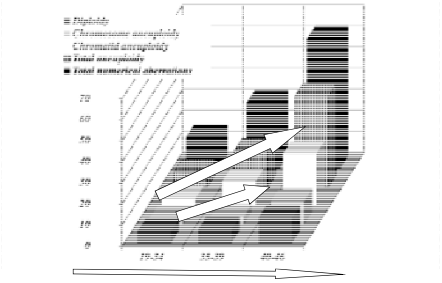
Increased transcripts for separase detected in aged human MII oocytes



Predivision: Precocious loss of cohesion between sister chromatid arms and centromeres

Cdk1 ?

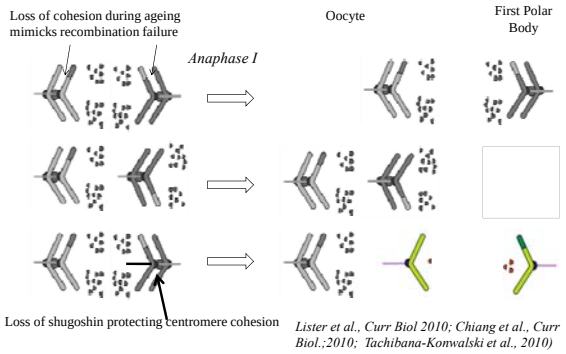
Classic cytogenetic studies on spread chromosomes of ‚spare'MII oocytes and PB 1 suggest that chromatid rather than chromosome aneuploidy increases substantially with increased age



Age

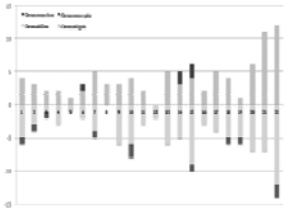
Pellestor et al., Hum Genet. 2003; 112(2): 195-203.

Precocious loss of cohesion predisposes to nondisjunction or predivision



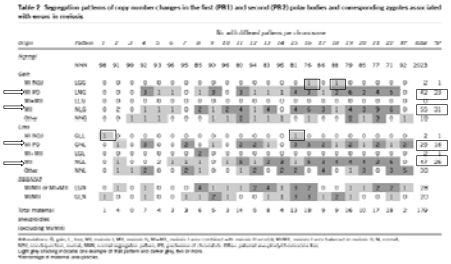
Lister et al., Curr Biol 2010; Chiang et al., Curr Biol.;2010; Tachibana-Konwalski et al., 2010)

Comparative genomic hybridisation suggests that aneuploidy in 1. PB mainly involves chromatids, increases with maternal age and involves several chromosomes



Gabriel et al., J Med Genet 2011

Comparative genomic hybridisation suggests that MI predivision and MII errors leading to gains or losses are comprising the vast majority of meiotic errors in maternal aneuploidies, in contrast to what is expected from trisomy data



Handyside et al., Europ. J Med Genet, 2012

Does stimulation contribute to conditions enhancing loss of chromosome cohesion?

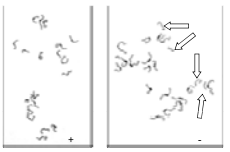
Does hyperstimulation increase susceptibilty to meiotic errors?

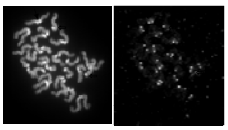
Human Reproduction Vol.22, No.4 pp. 998–1003, 2007
Advance Access publication January 4, 2007

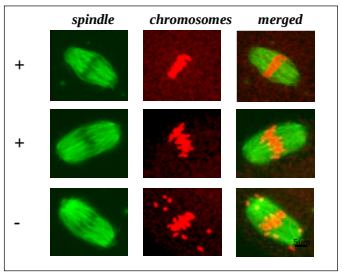
Milder ovarian stimulation for *in-vitro* fertilization reduces aneuploidy in the human preimplantation embryo: a randomized controlled trial

Esther B.Baart[1,2,4], Elena Martini[2], Marinus J.Eijkemans[3], Diane Van Opstal[1], Nicole G.M.Beckers[2], Arie Verhoeff[2], Nicolas S.Macklon[1] and Burt C.J.M.Fauser[1,2]

Can loss of cohesion become induced?
Sub-optimal culture media can induce predivision and congression failures







Relevance of redox regulation!

Conclusions:

The majority of extra chromosomes in trisomies are maternal and derived from meiosis I errors.

Failure to recombine gives rise to susceptibility to first (an second) meiotic errors.

PB analysis suggests that meiosis I errors are more common in young oocytes while meiosis II errors and predivision dramatically increase with age

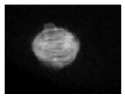
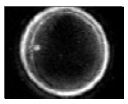
Loss of cohesion may be predominant factor in MI and MII errors in aged mouse oocytes.

However, up to age 37 there was no evidence for reduced cohesin protein in aged human oocytes (Garcia-Cruiz et al., Hum Reprod)

Cohesion complexes cannot be replaced in GV oocytes.
Can ‚environment' affect loss of cohesion and gene expression?

Cryopreservation of young oocytes option to preserve fertility to advanced ages.

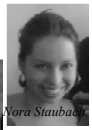
Thank you for your attention!


Improve oocyte quality and fertility

Gene Technology/Microbiology





Thorsten Seidel
University of Bielefeld

Francesca Pacchierotti
Univ Roma

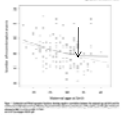
Linda Wordemann
University of Washington School of Medicine Seattle

Johan Smitz
FUB, Brussels

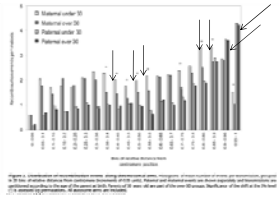
Carla Tatone
University of L'Aquila

Support by DFG (FOR 1041)

Currently disputed: Does recombination in normal eupolid conceptus increase or decrease with advanced maternal age?

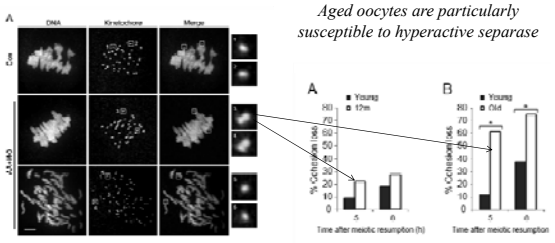


Less recombination until about 32 years; alteration in distal and subtelomeric regions of chromosomes - more susceptible to errors in aged oocyte (Hussin et al., Plos Genet. 2011)



Centromere ⟶ *Telomere*

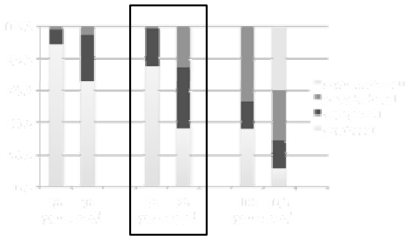
Hyperactive separase causes precocious release of centromere and arm cohesion of chromatids in mouse oocytes



Aged oocytes are particularly susceptible to hyperactive separase

Chiang et al., Biol. Reprod 2011

Evidence that disturbed cell cyle progression and altered gene expression may contribute to errors in chromosome segregation



Duncan et al. Biol Reprod 2009:
No difference in anaphase I between young & aged
Aged aneuploids not faster in anaphase I progression

But: aneuploidy appeared high in control:
8% (n= 37) in young and 35% (n=23) in aged oocytes! (Predivision??)

Loss of cell cycle control!

Unstimulated cycles at late diestrous; Modified M2

Eichenlaub-Ritter and Boll, Cytogenet Cell Genet. 1989;52(3-4):170-6.

Carbonyl Stress can contribute to mitochondrial dysfunction and spindle aberrations

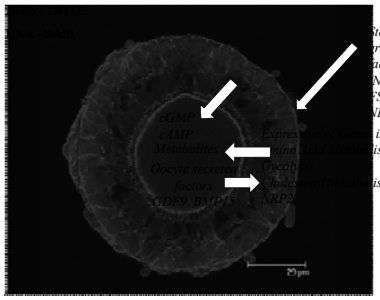
Accummulaion of serum toxic AGEs products and follicular fluid AGE compromises embryonic development and achievement of pregnancy by ART (Jinno et al. Hum Reprod. 2011).

These were associated with increased BMI, glucose, LDL and insulin but not with maternl age.

High risks for spindle aberrations and reduced developmental potential of oocytes appears related to follicular environment!

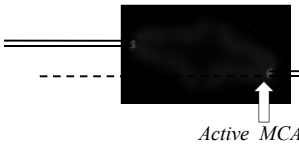
However, aging may affect follicles/oocytes differentially:
There is no age-related increase in aneuploidy in pig oocytes! (Hornak et al. PLOS One 2011)

Oocyte growth and regulation of expression depend on bi-directional signalling and oocyte secreted factors during meiotic arrest



Do disturbancs by ROS and altered metabolism affect chromosome segregation?

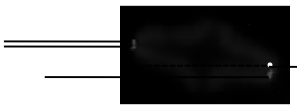
Young oocyte



Centromeres one unit; syntelic attachment favoured, Reductional separation likely

Active MCAK

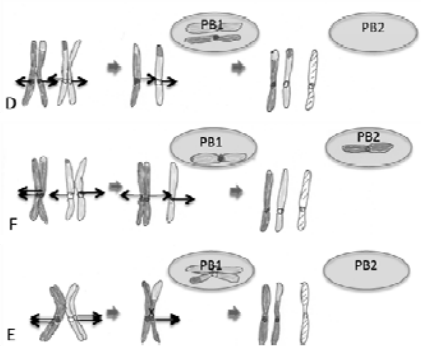
Aged oocyte



Partial loss of cohesion; Amphitelic and merotelic attachment facilitated, Equational separation likely

Reduced activity of MCAK: Lagging and segregation/ separation of Sister chromatids at meiosis I

Predivision at MI or MII at the origin of meiosis I and meiosis II errors



Conclusions II

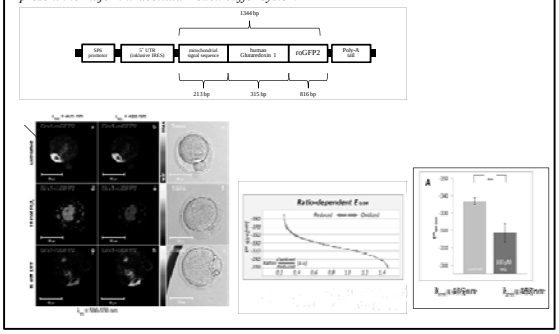
Certain recombination patterns predispose individual chromosomes to a meiotic error in an aged but not a young oocyte.

Processes at embryonic prophase I prior to birth influence susceptibility to errors but ‚hits' at later stages of meiosis are the major drivers of nondisjunction

Induction of second meiotic errors involving sister chromatids appear influenced by distribution of exchanges suggesting correlations between age and disturbance in the sequential segregation of homologs and chromatids in aged oocytes.

Exposure to methylglyoxal disturbs mitochondrial distribution and redox potential

Glutathione(GSH) and the GSH/GSSG system present the major intracellular redox buffer system

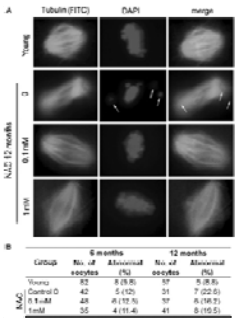


Most maternal contributions are already present in the fully grown, meiotically and developmentally competent oocyte

Postphoning age, and maintaining oocyte quality by improving defence against oxydative stress e.g. by antioxidant N-acetyl-L-cystein (Liu et al., Hum Reprod. 2012 in press):

Oocytes from NAC treated aged females had less congression failures.

Mice treated with low concentration (0.1 mM) of NAC had increased litter sizes at advanced ages compared with controls, expressed more sirtuins and had higher telomerase activity as well as extended telomere length.



Liu J et al. Hum. Reprod. 2012;humrep.des019

Conclusions I

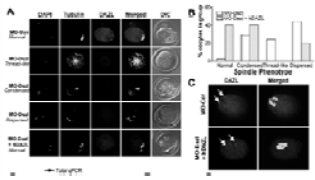
Absence of recombination increase susceptibility to first meiotic errors.

Absence of recombination also predispose to predivision and random segregation at MII.

Deficiencies/mutation in genes affecting recombination rate and localisation can contribute to increased or decreased risks for first meiotic errors.

Environmental exposures in utero can interfere with recombination patterns and affect risks for nondisjunction in offspring.

Differential Recruitment of mRNA is essential for normal spindle formation



Deficiency in DAZL causes aberrant spindles

DAZL induces translation of spindle proteins (e.g. TPX2) and is located at the poles

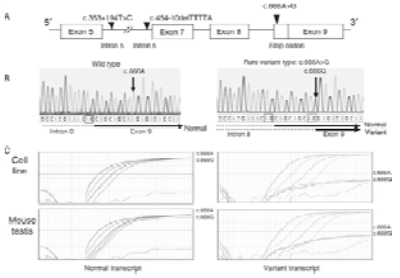
CPEB
⇩
DAZL
⇩
Spindle proteins

Chen et al., Genes & Dev, 2011

Fine tuning of expression/translational recruitment is mandatory for normal cell cycle progression and spindle formation in mammalian oocytes

Pairing and recombination

Genetic variations in the coding region of the SYCP3 gene that may contribute to meiotic disturbances and errors giving rise to oocyte aneuploidy.



Nishiyama et al. Mol. Hum. Reprod. 2011;17:266-271

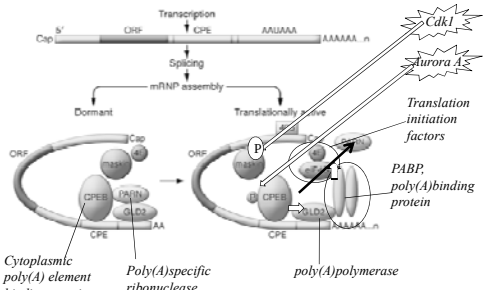
MHR

When do errors actually occur, meiosis I or meiosis II, before or after ovulation/fertilization?

Trisomy data suggest: The majority of extra chromosomes are maternal and derived from meiosis I errors (involving whole chromosomes such that the two maternally-derived chromosomes in the trisomy have centromeres that are from different homologues and not from not sister chromatids).

Trisomy 15: 90% meiosis I
Trisomy 16: 100% meiosis I
Trisomy 18: 37% meiosis I & 63% meiosis II
Trisomy 21: about 75% meiosis I, 25% meiosis II
Sex chromosomes: about 75% meiosis I, 25% meiosis Ii

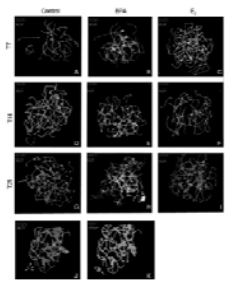
Protein (CPEB) phosphorylation can initiate dissociation of PARN, activation of poly(A)polymerase and interaction between poly(A)binding protein (PABP) and translation initiation factors

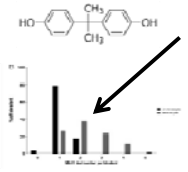


Gosden & Lee, The J Clin Investigation, 2010

Pairing and recombination

Altered meiotic progression/ SC formation and increased Mlh1 spots/recombination were detected in cultured human fetal ovarian pieces exposed to >10μM bisphenol A





Exposures in utero can disturb pairing/recombination and induce POI or predispose to nondisjunction at young ages

Brieño-Enríquez et al., Hum Reprod. 2011;26(10):2807-18