



## **12<sup>th</sup> International Conference on Prenatal Diagnosis and Therapy**

Budapest (Hungary), June 24-27, 2004

---

### **Organized by the**

I. Department of Obstetrics and Gynecology  
Semmelweis University

Baross utca 27

H-1088 BUDAPEST (Hungary)

**Postal Address:** H-1442 Budapest, P.O. Box 104

**Tel:** (+36-1) 267-1007, (+36-1) 317-6315

**Fax:** (+36-1) 317-6174

**E-mail:** [pz@noi1.sote.hu](mailto:pz@noi1.sote.hu)

**Internet:** [www.noi1.hu](http://www.noi1.hu)

**FINAL PROGRAM AND ABSTRACTS**

# Calendar of the International Conferences of the “International Society for Prenatal Diagnosis” (ISPD)

---

**Rapallo** (Italy), 1984

**Nottingham** (UK), 1985

**Chicago** (USA), 1987

**Athens**, (Greece), 1989

**Prague** (Czechoslovakia), 1990

**Milan** (Italy), 1992

**Jerusalem** (Israel), 1994

**Goa** (India), 1996

**Los Angeles** (USA), 1998

**Barcelona** (Spain), 2000

**Buenos Aires** (Argentina), 2002

**Budapest** (Hungary), 2004

**Fraccaro**, Marco

**Liu**, David

**Pergament**, Eugene

**Antsaklis**, Aris and **Metaxotou**, Catherine

**Macek**, Milan

**Brambati**, Bruno

**Zakut**, Haim

**Chakravarti**, Amit

**Platt**, Larry

**Fortuny**, Albert

**Gadow**, Enrique and **Otano**, Lucas

**Papp**, Zoltán

# Welcome to Budapest!

On behalf of the International Society for Prenatal Diagnosis (ISPD) it is my pleasure and honor to welcome you at the *12<sup>th</sup> International Conference on Prenatal Diagnosis and Therapy*.

The mission of ISPD is to advance the art and science of all aspects of fetal diagnosis. Now the Conference will provide an excellent opportunity to interact with world-class scientists and clinicians in

a multidisciplinary approach in the field of preimplantation diagnosis, fetal imaging, chromosome and DNA diagnosis, prenatal screening, fetal therapy and psychosocial and ethical issues. The scientific program aims to include the latest information for obstetricians and also for scientists interested in genetics and biochemistry as related to this increasingly important field within the speciality of fetal and maternal medicine.



Budapest – long known as the liveliest city in the former Eastern Bloc - is the capital of Hungary where one fifth of the population live and everything converges here: roads and rail lines, air travel, industry, commerce and culture, opportunities, wealth and power. The city has a history of revolutions and buildings, parks and avenues on a monumental scale. The River Danube – which is seldom really blue – determines basic orientation, with Buda on the hilly west bank and Pest on the plain east bank.

Surveying Budapest from the embankments or the bastions of Castle Hill, it's easy to see why the city was dubbed the "Pearl of the Danube". In short, Budapest is a city worthy of comparison with other great European capitals.

We are also proud to invite you to our nearly 200-year-old University Department, since 1896 located in the historical Baross Street building. In spite of the retained old-style exterior the requirements of the 21<sup>st</sup> century are fully met in its recently rebuilt interior. Ignac Semmelweis, the "Savior of Mothers" was the director of the Department between 1855 and 1865. His spirit and commitment for the sake of mothers and their unborn and newborn children are acknowledged worldwide, and are immortalized in the name: Semmelweis University in Budapest.

We look forward to giving you the unforgettable memory of an enlightening congress and the sincere, warm welcome of our people and city.

Yours sincerely,

Professor Zoltán Papp  
President of the Congress

# ISPD Officers and Board Members

---

<b>President:</b>	<b>Rodeck</b> , Charles (London, UK)
<b>Past presidents:</b>	<b>Ferguson-Smith</b> , Malcolm (Cambridge, UK) 2000-2002 <b>Simpson</b> , Joe Leigh (Houston, USA) 1998-2000
<b>Secretary:</b>	<b>Bianchi</b> , Diana (Boston, USA)
<b>Treasurer:</b>	<b>Gadow</b> , Enrique (Buenos Aires, Argentina)
<b>Board members:</b>	<b>Fortuny</b> , Albert (Barcelona, Spain) <b>Holzgreve</b> , Wolfgang (Basel, Switzerland) <b>Pergament</b> , Eugene (Chicago, USA) <b>Shulman</b> , Lee (Chicago, USA) <b>Suzumori</b> , Kaoru (Nagoya, Japan) <b>Wapner</b> , Ronald (Philadelphia, USA)
<b>Board members-elect:</b>	<b>Johnson</b> , Jo-Ann (Toronto, Canada) <b>Lo</b> , Dennis (Hong-Kong, China) <b>Wald</b> , Nicholas (London, UK)

---

## Local Organizing Committee

<b>Chairman:</b>	<b>Papp</b> , Zoltán (Budapest, Hungary)
<b>Secretary General:</b>	<b>Hupuczi</b> , Petronella (Budapest, Hungary)
<b>Secretary:</b>	<b>Bán</b> , Zoltán (Budapest, Hungary)
<b>Members:</b>	<b>Csaba</b> , Ákos (Budapest, Hungary) <b>Fekete</b> , Tibor (Budapest, Hungary) <b>Belics</b> , Zorán (Budapest, Hungary) <b>Than</b> , Nándor (Budapest, Hungary) <b>Halmos</b> , Amrita (Budapest, Hungary) <b>Lázár</b> , Levente (Budapest, Hungary) <b>Nagy</b> , Gyula Richárd (Budapest, Hungary)

# CONGRESS INFORMATION

## Venue

The venue of the Congress is the Central Building of the Semmelweis University (NET), Nagyvárad square 4, Budapest.

The building was built in the late 70's as a typical representation of modern architecture of the era. The 23-storeyed Central Building is the highest building of the city. The registration and Congress Information Desk in the Central Building of the Semmelweis University will be open on Thursday (June 24) from 12.00 until 22.00, on Congress days from 7.00 until 19.00, and on Sunday (June 27) from 7.00 until 14.00.



## Presentations

---

The program consists of keynote lectures on current topics in the main areas of fetal medicine presented by renowned specialists of the field and free communications in the form of posters presented by participants.

### Oral presentations

The invited keynote lectures will be 15 minutes long followed by a 3 minutes discussion.

### Posters

Free communications have been accepted in the form of posters. Posters are on display from 08.00 AM until the end of the daily program.

## Congress language

---

The official language of the Congress is English. There is *no* simultaneous translation.

## Registration fee

---

Before February 15, 2004	EURO 600
After February 15, 2004	EURO 700
Accompanying person	EURO 300
Students* and midwives	EURO 200

\*Current student status to be certified by official University statement.

### The registration fee of participants includes:

- Final Program and Abstracts
- ISPD membership fee for 2004 and one year's subscription of the journal Prenatal Diagnosis (Wiley)
- Lunch and coffee
- Admission to all scientific sessions (plenary sessions, posters, exhibition)
- Participation in the Get Together Party, Ballet Performance and Boat Trip on the Danube.

The reduced price for *students and midwives* includes scientific programs and meals but does not include social programs.

The *accompanying person's* fee covers admission to the Get Together Party, Ballet Performance and Boat Trip on the Danube.

# AUDIOVISUAL MATERIAL

---

## Posters

The maximum width of the posters should be 120 cm and height 160 cm.

## Projection and Technical Settings

*PC projection* will be available. To use this facility, please prepare your presentation using a **Microsoft® Power Point format**. Maximum Resolution: XGA (1024 X 768 pixel). **CD-ROM, ZIP 100/250-, or floppy disk** is preferred. It is essential that you load and view your presentation at the **Slide Preview Desk** preferably in the morning of the day your talk is scheduled, but **not later than 1 hour before your session starts**. Your file will be copied to the hard-drive of the computer, in order to provide a quick and reliable projection. Files will be handled confidentially and deleted after the sessions.

## LUNCH

Lunch is provided for participants on Friday and Saturday.

## COFFEE

Coffee service is available free of charge during the Congress.

## BADGES

You are kindly asked to wear your congress badge during the entire meeting. The badge is needed to enter the Congress area and gives direct access to all scientific activities.

# EXHIBITORS

---

Acom Healthcare Europe  
75 Avenue Parmentier  
75011 Paris, France

Applera Hungary Ltd.  
Hermina u. 17  
1146 Budapest, Hungary

FertiCad Ltd.  
Késmárki u. 6  
1118 Budapest, Hungary

John Wiley & Sons Ltd.  
Chichester, West Sussex  
PO19 8SQ, UK

Oxford Bio-Innovation Ltd. - DSL  
77 Heyford Park  
Upper Heyford, Oxfordshire  
OX25 5HD, UK

Perkin-Elmer Life and Analytical Sciences  
Wallac Oy, P.O. Box 10  
20101 Turku, Finland

Plasmagene Ltd.  
16 Ice House Street  
Central Hong Kong

Schering Ltd.  
Szépvölgyi út 35-37, 1037  
Budapest, Hungary

# VISITING THE I. DEPARTMENT OF OBSTETRICS AND GYNECOLOGY, SEMMELWEIS UNIVERSITY

The participants of the Congress are kindly invited to visit the 190-year-old University Department, which has been located in the historical building in Baross street for 110 years. In the last decade of its history the building was renewed and modernized, providing the reputable atmosphere of the past added to the latest technical and infrastructural facilities. The units of the Department are as follows:

## Major Outpatient Units:

- Pregnancy Care Unit
- Gynecological Outpatient Clinic
- Ultrasound Diagnostic Laboratory
- Family-planning Unit
- Pediatric Gynecology Outpatient Clinic
- Menopause Outpatient Clinic
- Genetic Counseling

**Operating Theatre and Postoperative Care Unit**  
**Delivery Room and Post-Partum Ward**

**Neonatal Intensive Care Unit**  
**Assisted Reproduction and Endocrinology Unit**

**Pregnancy Pathology Ward**  
**Gynecology Ward**

## Laboratories:

- Chemical Laboratory
- Endocrine Laboratory
- Cytogenetic and Molecular Genetic Laboratory
- Histopathology, Embryo- and Fetopathology and Cytology Laboratory



All participants are warmly welcome to visit the Department during the Congress. Guided tours can be scheduled at the Registration Desk.

# SOCIAL PROGRAM

## **THURSDAY (JUNE 24) 19.30 – 22.00**

**Get Together Party** on the site of the Congress in the Central Building of Semmelweis University.

## **FRIDAY (JUNE 25) 19.00 – 22.00**

**Ballet Performance** at the Budapest Opera House  
Mendelssohn's Midsummer Night's Dream choreographed by László Seregi.

Bus transport to the Opera House will be provided from both the Hotel Korona and the Congress venue.  
*Buses leave at 18.00!*



## **SATURDAY (JUNE 26) 19.30 – 23.00**

**Boat Trip and Dinner on the Danube**

Bus transport to the Danube will be provided from both the Hotel Korona and the Congress venue.  
*Buses leave at 19.00!*



# USEFUL INFORMATION

---

Hungary is situated in Central Europe, in the Carpathian Basin. It covers an area of 93,000 square kilometers and has a population of approximately 10.5 million. Budapest is the capital of the country.

Budapest can be reached from all parts of the world. Hungary has 8 highways, all of which are linked to the European road network. The international airport of Budapest, Ferihegy is located 15 km from the center and is a major gateway to most European and international cities. From the airport convenient and quick transfer is provided to all hotels for a reasonable price.

**For the congress participants the Organizing Committee organized minibus transport between the Conference Venue and the Hotel Mercure Korona in every 15 minutes.**

Budapest has no central railway station, but visitors may arrive at one of the three major railway stations, the Western, Southern, or Eastern stations. Budapest can also be reached by boat via the river Danube.

Budapest with its wealth of historic monuments, more than 30 museums, galleries and dynamic cultural life is an experience. The busy life of the city, hospitality of the residents, small inns and luxury restaurants add to the special charming atmosphere of the Hungarian Capital.

## Public Transport

Public transportation in Budapest is well organized with its 3 underground lines and numerous bus and tram lines. Charges for all facilities are equal: 140 HUF (approx. 0.5 EURO) for a one-way-ticket. A week-ticket costs 2600 HUF (approx. 10 EURO). All parts of the city can be easily reached using the public transport facilities.

## Taxi

More than 15,000 taxis are currently operating on the streets of the city. It is highly recommended that you order a taxi by phone instead of hailing one on the streets. Currently, the average taxi fare is 260 HUF (1 EURO) / km.

## Parking

Open-air parking facilities are limited in Budapest. Free-of-charge parking facilities are provided in the courtyard of the Central Building of the Semmelweis University. It is recommended that you park your cars in the hotels, since the Congress is only a short walking distance and public transport is well organized.

## Banking Facilities / Exchange

A great number of banks and exchange bureaus will help you with your requirements.

## Currency

Hungarian currency is the *Hungarian Forint (HUF)*. At the present time approx. 260 HUF is equal to 1 EURO. Exchange of your currency is widely provided in all hotels and banking facilities. Major credit cards are widely accepted.

## Electric Supply

Electricity supply in Hungary is 220/230 Volts, AC 50 Hz. Adapters for plugs may be necessary.

## **Insurance and Liability**

The organizers will not be responsible for individual medical, travel or personal expenses and delegates are advised to take out their own personal insurance policy.

## **Medical Service**

Available in the hotels.

## **Shopping**

You will find hundreds of shops in the city ranging from small boutiques to big warehouses. Shops usually open until 18.00 in Hungary.

## **Tipping**

Is generally expected in restaurants. The recommended amount is 10 percent of the bill.

## **Postal Service**

Post offices are generally open from Monday to Friday 8.00 to 19.00 and on Saturdays to 13.00.

## **Telephone, Fax and Internet**

Area code for Hungary is (36), and (1) for Budapest. Budapest phone numbers have 7 digits. Public phones operate with coins or phone card.

## **Emergency Telephone Numbers:**

Ambulance	104
Police	107
Fire	105
Using a mobile phone	112

## **MALÉV, Hungarian Airlines**

The official airline for the Congress is MALÉV. Its worldwide affiliates will meet your travel requirements. We advise all Congress participants to reserve their flight as soon as possible.

## **Rental Cars**

Car rental companies have their offices at the Airport and in the city. Please, inquire at your hotel.

## **Congress Bus Transport**

A minibus shuttle will be provided between the Congress venue and the Hotel Korona. A subway line also runs between the Hotel Korona and the Congress venue (three stops). A ticket costs HUF 140.

## IMPORTANT ADDRESSES

---

**Congress Secretariat** (*before June 24, 2004*)

I. Department of Obstetrics and Gynecology  
Semmelweis University  
Baross u. 27. 1088 Budapest, Hungary

**Tel:** (+36-1) 267-1007 or (+36-1) 317-6315

**Fax:** (+36-1) 317-6174

**Email:** [pz@noi1.sote.hu](mailto:pz@noi1.sote.hu)

**Congress Venue** (*June 24 - 27, 2004*)

Central Building of the Semmelweis University ("NET")  
Nagyvárad tér 4. 1089 Budapest, Hungary  
Tel: (+36-1) 210-2940 Ext. 6290

<http://www.wiley.com/ispd>

<http://www.noi1.hu>

## BOARD MEETINGS

---

- **Board Meeting of the Preimplantation Genetic Diagnosis International Society (PGDIS):**  
Thursday (June 24, 2004) 14.00 - 18.00
- **Editorial Board Meeting of Prenatal Diagnosis:**  
Friday (25 June, 2004) 12.00 - 14.00
- **Board Meeting of the International Society for Prenatal Diagnosis (ISPD):**  
Saturday (26 June, 2004) 07.00 - 07.40
- **ISPD AGM:**  
Saturday (26 June, 2004) 07.40 - 08.00

The meetings will be held at the Conference Venue.

## Friday, 25 June

---

### 09.00 Opening

Professor **Papp Z**, President of the Conference  
Dr. **Kökény M**, Minister of Health in Hungary  
Professor **Tulassay T**, Rector of the Semmelweis University  
Professor **Rodeck C**, President of the ISPD

### 09.15-12.00 Preimplantation genetic diagnosis

Chair: **Simpson JL (Houston, USA) and Verlinsky Y (Chicago, USA)**

#### Oral presentations

1. Simpson JL (Houston, USA): Preimplantation genetic diagnosis
2. Kuliev A, Cieslak J, Zlatopolsky Z, Illkevitch Y, Kirilova I, Verlinsky Y (Chicago, USA): Origin of aneuploidies in preimplantation embryos
3. Verlinsky Y, Rechitsky S, Kuliev A (Chicago, USA): Preimplantation diagnosis for single gene defects and its application to preimplantation HLA typing
4. Gianaroli L, Magli MC, Ferraretti AP (Bologna, Italy): The value of preimplantation genetic diagnosis as a clinical prognostic tool

#### Coffee Break

5. Munne S, Escudero T, Fischer J, Colls P, Zheng X, Maria O, Cohen J (New Haven and West Orange, USA): PGD to reduce spontaneous abortions in translocation carriers and patients with recurrent miscarriages
6. Sermon K (Brussels, Belgium): Increasing efficacy and safety of preimplantation genetic diagnosis for monogenic diseases. One cell versus two cell biopsy: a difficult choice
7. Wells D, Bermudez M, Steuerwald N, Chu L, Weier U, Cohen J, Munne S (West Orange and Hoboken, USA): Microarrays for analysis and diagnosis of preimplantation embryos
8. Harper J (London, UK): Future developments in preimplantation genetic diagnosis
9. Khatamee M, Horn S, Weseley A, Farooq T, Jaffe S, Jewelwicz R (New York, USA): A controlled study for gender selection using swim-up separation

#### Poster presentations (Friday, 25 June)

10. Daphnis D, Harper J, Jerkovic S, Geyer J, Craft I, Delhanty J (London, UK): Detailed FISH analysis on day 5 human embryos reveals the mechanisms leading to mosaic
11. Fragouli E, Conn CM, Cupisti S, Wells D, Whaley K, Mills JA, Faed MJW, Delhanty JDA (London and Dundee, UK, New Jersey, USA, Erlangen-Nürnberg, Germany): Molecular cytogenetic investigations of aneuploidy: FISH and CGH analysis of human oocytes and polar bodies
12. Garda AL, Martínez S, Gómez E, Martínez MC, Pérez I, Amorocho B, Landeras J, Ballesteros A (Murcia and San Juan, Spain): Expression of platelet-activating factor acetylhydrolase (Ib) and PAF-receptor in the human oocyte
13. Malcov M, Frumkin Z, Schwartz T, Mey-Raz N, Amit A, Ben Yosef D, Azem F, Lessing JB, Yaron Y (Tel Aviv, Israel): Preimplantation genetic diagnosis for Ashkenazi Jewish genetic disorders
14. Martín J, Rubio C, Mercader A, Simón C, Remohí J, Pellicer A (Valencia, Spain): Preimplantation genetic diagnosis experience for single-gene diseases: initial results
15. Mateu E, Rubio C, Rodrigo L, Serrano C, Remohí J, Pellicer A (Valencia, Spain): FISH analysis in sperm samples from patients with recurrent hydatiform moles
16. Pérez I, Rubio C, Rodrigo L, Mercader A, Mateu E, Ballesteros A, Landeras J, Remohí J, Pellicer A (Murcia and Valencia, Spain): PGD in advanced maternal age: incidence of chromosomal abnormalities and pregnancy outcome
17. Rodrigo L, Mateu E, Mercader A, Buendía P, Remohí J, Pellicer A, Rubio C (Valencia, Spain): Rescue of false monosomies in a PGD program using subtelomeric probes

# Friday, 25 June

---

## 14.00-17.00

### Fetal cells and nucleic acids in maternal circulation

Chair: Bianchi D (Boston, USA) and Pertl B (Graz, Austria)

---

#### Oral presentations

- 18.** Bianchi DW, Larrabee PB, Wataganara T, Khosrotehrani K, Johnson KL (Boston, USA): Fetomaternal trafficking of cells and nucleic acids: diagnostic and therapeutic applications
- 19.** Bischoff FZ (Houston, USA): Cell-free fetal DNA in maternal blood: Molecular structure and enrichment approaches
- 20.** Kan YW, Feng D, Cheung MC (San Francisco, USA): Fetal cells in maternal circulation for prenatal diagnosis
- Coffee Break**
- 21.** Lo YMD (Hong Kong, China): Recent developments in the biology and diagnostic applications of fetal nucleic acids in maternal plasma
- 22.** Hultén M (Coventry, UK): SAFE - an EC network of excellence on non-invasive prenatal diagnosis
- 23.** Oudejans CBM, Go ATJJ, Visser A, Mulders MAM, Blankenstein MA, van Vugt JMG (Amsterdam, The Netherlands): Detection of chromosome 21-encoded mRNA of placental origin in maternal plasma
- 24.** Lázár L, Bán Z, Harmath Á, Papp Z (Budapest, Hungary): The presence of maternal DNA in peripheral blood of newborn infants

#### Poster presentations (Friday, 25 June)

- 25.** Chim SSC, El-Sheikhah A, Tsui NBY, Ng EKO, Chiu RWK, Chan KCA, Tong Y, Hogg M, Bindra R, Leung TN, Lau TK, Nicolaides KH, Lo YMD (Hong Kong, China and London, UK): Cell-free fetal RNA in maternal circulation as a new tool for the non-invasive detection of aneuploid pregnancies
- 26.** Cirigliano V, Bulmer J, Cioni R, Sole F, Costa C, Adinolfi M (Barcelona, Spain, Newcastle-upon-Tyne, UK, Firenze, Italy and London, UK): Non invasive prenatal diagnosis by laser microdissection of HLA-G positive trophoblastic cells in transcervical samples and QF-PCR
- 27.** Katz-Jaffe M, Mantzaris D, Cram D (Melbourne, Australia): DNA identification of fetal cells isolated from cervical mucus: potential for early non-invasive prenatal diagnosis
- 28.** Lau ET, Kwok YK, Chui DHK, Luo HY, Leung KY, Lee CP, Lam YH, Tang MHY (Hong Kong, China and Boston, USA): Analysis of fetal erythrocytes in maternal blood for prenatal detection of Hb Bart's disease
- 29.** Lázár L, Bán Z, Nagy B, Beke A, Rigó J, Papp Z (Budapest, Hungary): Sex determination by the detection of SRY region with real-time PCR in maternal plasma
- 30.** Levy-Mozziconacci A, Ponceillé B, Muller FF, Roux F, Chaudet H, Mancini J, D'Ercole C, Boubli L, Gabert J (Marseille and Paris, France): Method for standardization of fetal DNA detection and quantification from maternal blood / preliminary stage of an international multicentric study
- 31.** Mavrou A, Kolialexi A, Souka A, Pilalis A, Kavalakis Y, Antsaklis P, Kanavakis E, Antsaklis A (Athens, Greece): First trimester NRBC count in maternal circulation: correlation with Doppler ultrasound studies
- 32.** Nagy GyR, Bán Z, Sipos F, Oroszné Nagy J, Beke A, Papp Z (Budapest, Hungary): Our first steps in detecting fetal cells in the maternal circulation for prenatal diagnosis
- 33.** Zimmermann S, Hollmann C, Stachelhaus S (Langenhagen, Germany): Development of monoclonal antibodies for the differentiation of fetal and adult erythroid blood cells

## Saturday, 26 June

---

### 09.00-12.00 Prenatal diagnosis of genetic disorders and prenatal infections

Chair: Rodeck C (London, UK) and Pergament E (Chicago, USA)

---

#### Oral presentations

- 34. Fiorentino F (Rome, Italy): Prenatal diagnosis of single gene disorders
- 35. Kleijer WJ (Rotterdam, The Netherlands): Prenatal diagnosis of genetic metabolic disorders
- 36. Old J (Oxford, UK): 20 years experience of prenatal diagnosis of hemoglobin disorders by DNA analysis
- 37. Otano L (Buenos Aires, Argentina): Prenatal diagnosis and treatment of congenital adrenal hyperplasia due to 21-OH-hydroxylase deficiency

#### Coffee Break

- 38. Pergament E, Fiddler M (Chicago, USA): The sooner we know...?
- 39. Stipoljev F, Kurjak A (Zagreb, Croatia): Prenatal ultrasound screening of most common hereditary disorders in low-risk pregnant women population
- 40. Philip J (Copenhagen, Denmark): Fetal loss, clubfoot deformity and early amniocentesis: A comparison of early amniocentesis and late chorionic villus sampling in an international randomized study
- 41. Nicolini U (Milano, Italy): Maternal and fetal lymphocyte subpopulations in CMV infection
- 42. Ville Y (Poissy, France): Prenatal diagnosis of congenital infections

#### Poster presentations (Saturday, 26 June)

- 43. Csikós M, Rác E, Benkő R, Bóna A, Bán Z, Beke A, Papp Z, Horváth A, Kárpáti S (Budapest, Hungary): Prenatal diagnosis of Herlitz junctional epidermolysis bullosa
- 44. Hernandez SS, Suy A, Pisa S, Borrell A, Coll O (Barcelona, Spain): Prenatal diagnosis among HIV infected pregnant women
- 45. Mahoney M, Cronister A, DiMaio M, Donnenfeld A, Hallam S (New Haven, USA): Fragile X carrier screening in the prenatal genetic counseling setting in the United States
- 46. Pikó H, Balog J, Bán Z, Mayer P, Horváth R, Karcagi V (Budapest and Hódmezővásárhely, Hungary): Prenatal diagnosis of three Hungarian families affected by facioscapulohumeral dystrophy
- 47. Quadrelli R, Vaglio A, Diaz A, Lemes A, Larvandaburn M (Montevideo, Uruguay): Prenatal diagnosis of cerebro-costo-mandibular syndrome (CCMS)
- 48. Shulman LP, Yates C, Hammond C (Chicago, USA): Markedly elevated amniotic fluid alpha-fetoprotein associated with fetal Klippel-Trenaunay-Weber syndrome
- 49. Stipoljev F, Hafner T, Sertic J, Kos M, Rukavina-Stavljenic A (Zagreb, Croatia): The incidence of cystic fibrosis in second-trimester fetuses with hyperechoic bowel
- 50. Tarnawa V, Herczegfalvi Á, Tímár L, Hajdú K, Tóth A, Siska É, Karcagi V (Budapest, Hungary): Ten years experience on prenatal diagnosis of spinal muscular atrophy in Hungarian families. An overview of 87 cases

## Saturday, 26 June

---

### 14.00-18.00 Prenatal screening and diagnosis of chromosome disorders

Chair: Evans M (New York, USA) and Katz M (White Plains, USA)

---

#### Oral presentations

- 51. Wapner R (Philadelphia, USA): First-trimester screening for trisomy 21 and 18
- 52. Fortuny A, Borrell A, Casals E (Barcelona, Spain): First trimester two-step combined test for aneuploidy screening
- 53. Canick J (Providence, USA): First and second trimester serum markers: results of the FASTER trial
- 54. Wald N (London, UK): Comparison of different tests on antenatal screening for Down syndrome
- 55. Bán Z (Budapest, Hungary): Rapid diagnosis of aneuploidies by QF-PCR. Experience on 5000 samples

#### Coffee Break

- 56. Chitty LS (London, UK): Aneuploidy exclusion or full karyotyping: Is fetal ultrasound useful?
- 57. Wolstenholme J (Newcastle upon Tyne, UK): Mosaicism in CVS and embryos: linking CPM with its origins
- 58. Borrell A, Cuckle H (Barcelona, Spain and Leeds, UK): Ductus venosus assessment for trisomy 21 screening in addition to the combined tests
- 59. Chasen ST, McCollough LB, Chervenak FA (New York, USA): Is nuchal translucency screening associated with different rates of invasive testing in an older obstetric population?
- 60. Antsaklis A (Athens, Greece): Soft markers for aneuploidy in second trimester of pregnancy

#### Poster presentations (Saturday, 26 June)

- 61. Aiello H, Otano L, Igarzábal L, Gadow EC (Buenos Aires, Argentina): Nuchal translucency and gestational age
- 62. Beke A, Joó JG, Csaba Á, Papp Cs, Tóth-Pál E, Bán Z, Belics Z, Fekete T, Barakonyi E, Papp Z (Budapest, Hungary): Ultrasound minor and major anomalies detected in fetuses with aneuploidies in second trimester
- 63. Belics Z, Csabay L, Szabó I, Beke A, Fekete T, Halmos A, Papp Z (Budapest, Hungary): Prenatal sonographic measurement of the fetal iliac angle during the second trimester of pregnancy
- 64. Chitayat D, Huang T, Summers AM (Toronto, Canada): Is increased AF-AFP associated with higher risks of fetal chromosomal or structural abnormalities, or adverse pregnancy outcomes?
- 65. de Pater J, Kroes HY, Dorland M, Verschuren M, van Oppen C, Albrechts JCM, Engelen JJM (Utrecht and Maastricht, The Netherlands): Mosaic trisomy (8)(p22p23) in a fetus caused by a supernumerary marker chromosome without alphoid sequences
- 66. Faas B, Kooper A, van den Berg P, van Ravenswaay C, Hamel B, Geurts van Kessel A, Smits A (Nijmegen, The Netherlands): The diagnostic impact of replacing conventional prenatal karyotyping by selective molecular testing
- 67. Ginsberg N, Tsukerman G, Sibul M, Chmura M, Shulman L, Verlinsky Y (Highland Park and Chicago, USA): Association between very low level of PAPP-A in maternal serum and confined placental mosaicism for chromosome 3
- 68. Halliday J, Muggli E (Parkville and Melbourne, Australia): Increasing prenatal detection rates of Down syndrome in Victoria 1992-2002
- 69. Huang T, Alberman E, Wald N, Summers AM (Toronto, Canada and London, UK): Maternal serum screening in the second trimester can identify the majority of triploid pregnancies

- 70.** Huang T, Owolabi T, Summers AM, Meier C, Wyatt PR (Toronto, Canada): The association between markers levels and the risk of spontaneous fetal loss as in a second trimester maternal serum screening population
- 71.** Lee CP, Tang M, Tang R, Tse HY, Woo H, To WK, Wong SF, Wong K, Lam YH (Hong Kong and Macau, China): Acceptability of first and second trimester screening for fetal Down's syndrome. Interim results from a demonstration trial
- 72.** Lefort G, Blanchet P, Chaze A-M, Vago P, Lochu P, Lallaoui H, Pinton A, Pellestor F, Sarda P, Claustres M (Montpellier, Clermont Ferrand, La Rochelle and Toulouse, France): Prenatal molecular cytogenetic diagnosis of partial trisomy 1 due to neocentromere formation
- 73.** Leung WC, Lau ET, Tang MHY (Hong Kong, China): Can rapid aneuploidy screening (RAS) replace traditional karyotyping for women with amniocenteses performed for advanced maternal age?
- 74.** Lewis S, Cullinane F, Carlin J, Chitty L, Bishop A, Marteau T, Halliday J (Melbourne, Australia and London, UK): A comparison of Australian and UK obstetricians' and midwives' preferences for screening tests for Down syndrome: a conjoint analysis study
- 75.** Macek M, Hajek P, Vilimova S, Potuznikova P, Simandlova M, Vlk R, Havlovicova M, Hladikova M (Prague, Czech Republic): PAPP-A / PROMBP in prenatal screening of severe fetal development disorders and postnatal detection of acute coronary disease
- 76.** Martínez MA, Goncé A, Borrell A, Mercadé I, Casals E, Fortuny A, Cararach V (Barcelona, Spain): First trimester screening for Down syndrome in twin pregnancies using either nuchal translucency or the combined test
- 77.** Nieuwint AWM, de Pater JM, Madan K, (Amsterdam and Utrecht, The Netherlands): To what extent can the MLPA technique replace standard chromosome analysis?
- 78.** Olde Weghuis D, van Ravenswaal C, de Leeuw N, Creemers J, de Vries B, Sistermans E (Nijmegen, The Netherlands): Unexpected malformations in a female fetus with a deletion Xp: demonstration of a cryptic translocation by MLPA
- 79.** Oroszné Nagy J, Bán Z, Nagy GyR, Lázár L, Papp Z (Budapest, Hungary): Recurrent cases of uniparental disomy and trisomy 21 in a family
- 80.** Otano L, Aiello H, Igarzábal L, Matayoshi T, Gadow EC (Buenos Aires, Argentina): Performance of first trimester nasal bone evaluation as a marker for Down syndrome
- 81.** Perez Iribar MM, Cusi V, Aguayo A, Zabala T, Vela A (Barcelona, Spain): Cytogenetics and pathology in the evaluation of spontaneous abortion
- 82.** Pérez M, Borrell A, Farre MT, Borobio V, Figueras F, Puerto B, Fortuny A, Cararach V (Barcelona, Spain): The 11-14 week scan. Screening for structural and chromosomal fetal defects
- 83.** Pescia G, Ditesheim PJ, Faway CH, Nguyen The H, Schmid D, Brioschi PA (Lausanne, Switzerland): First trimester screening for Down syndrome in private practice combining biochemical markers and nuchal translucency measurements. Results of 11.000 consecutive pregnancies
- 84.** Sándor J, Szunyogh M, Métneki J, Siffel Cs (Budapest, Hungary and Atlanta, USA): Monitoring of geographical inequalities of Down syndrome occurrence in Hungary and its application in prenatal screening related problems identification
- 85.** Schielen PCJI, Elvers LH, Loeber JG (Bilthoven, The Netherlands): Experience with first trimester screening in the Netherlands
- 86.** Snijder S, Knegt AC, Muller MA, Verjaal M, Bilardo CM (Amsterdam, The Netherlands): Structural chromosomal aberrations in relation to increased nuchal translucency

- 87.** Stejskal D, Brouckova M, Brestak M, Louckova M (Prague, Czech Republic): Can trisomy 21 selective amnio-PCR be part of Down syndrome screening?
- 88.** Suijkerbuijk RF, Sikkema-Raddatz B, Dijkhuizen T, Dijkhuis J, van der Veen AY, Bouman K, Gerssen-Schoorl KBJ (Groningen, The Netherlands): Evaluation of multiplex ligation-dependent probe amplification as a means to rapidly detect unbalanced abnormalities of all chromosomes in prenatal cytogenetic analysis
- 89.** Suzumori K, Kondo Y, Sonta S, Tanemura M, Sugiura M (Nagoya, Japan): Maternal uniparental disomy of chromosome 16 in a case of spontaneous abortion
- 90.** Szigeti Zs, Bán Z, Papp Cs, Tóth-Pál E, Beke A, Joó JG (Budapest, Hungary): Fetal cytogenetic analysis of fetuses conceived by intracytoplasmic sperm injection
- 91.** To WWK, Chan AMY, Mok KM (Hong Kong, China): The use of amniocentesis and QF-PCR techniques for rapid karyotype diagnosis in late second trimester and third trimester to replace cordocentesis
- 92.** Tsukerman G, Pribushenya O, Krapiva G, Lishtvan L, Venchikova N, Shreder S, Kovalev S, Solovyeva I, Savenko L, Novikova I, Kirillova I (Chicago, USA and Minsk, Belarus): First trimester screening of fetal structural anomalies in a general obstetric population
- 93.** Wyatt PR, Owolabi T, Summers AM, Meier C, Huang T (Toronto, Canada): Age specific risk of fetal loss observed in a second trimester serum screening population

## Sunday, 27 June

### 08.00-10.30 Fetal diagnosis and therapy

Chair: Rodeck C (London, UK) and Holzgreve W (Basel, Switzerland)

#### Oral presentations

- 94.** Evans M (New York, USA): Framing decision in multiple pregnancies and other complex situations
- 95.** Stekelenburg-de Vos S, Ursem NTC, Wladimiroff JW, Groenendijk BCW, Poelmann RE (Rotterdam and Leiden, The Netherlands): Hemodynamics and heart function in the chick embryo during development of cardiovascular malformations
- 96.** Queenan J (Washington, USA): Rhesus and other isoimmunizations: an update
- 97.** Fisk N (London, UK): Stem cells for fetal diagnosis and therapy
- 98.** Argibay P, Lorenti A, Barbich M, Elias D, Hyon SH, Farias P (Buenos Aires, Argentina): In-utero hepatocyte xenotransplantation from pig to lamb
- 99.** Pertl B, Fast C, Eder M, Resch B, Urlesberger B, Haas J (Graz, Austria): Cystic periventricular leucoencephalomalacia in the preterm infant
- 100.** Sepulveda W (Santiago, Chile): Treatment of acardiac twinning
- 101.** Rodeck C (London, UK): Developments in fetal gene therapy

#### Coffee Break

#### Poster presentations (Sunday, 27 June)

- 102.** Cobo T, Martinez-Zamora MA, Borrell A, Puerto B, Martinez-Crespo JA, Borobio V, Botet F, Nadal A, Albert A, Cararach V, Vanrell JA (Barcelona, Spain): False-positives in the prenatal ultrasound screening for fetal malformations
- 103.** Csaba Á, Bán Z, Joó JG, Lázár L, Papp Z (Budapest, Hungary): How painful is amniocentesis?

- 104.** De Clippel KAJ, Wijman MJNC, Struijk PC, Wladimiroff JW, Steegers EAP (Rotterdam, The Netherlands): Longitudinal volume measurements of the human secondary yolk sac using three dimensional ultrasound: preliminary results
- 105.** Dienes J, Nagy G, Kékes K (Miskolc, Hungary): Uterine artery Doppler velocimetry in low-risk nulliparous women and in pregnancies complicated by diabetes mellitus (type 1 and 2)
- 106.** Garamvölgyi Z, Krasznai I, Hidvégi J, Rigó J Jr (Budapest, Hungary): Perinatal outcomes regarding to the risk factors and the efficacy of the treatment of diabetes mellitus
- 107.** Garcia M, Argibay P, Hidalgo A, Barbich M, Vieiro M, Hyon SH, Farias P (Buenos Aires, Argentina): In utero pancreatic islet xenotransplantation develops an autoimmune diabetes like process
- 108.** Groenewout M, Wijman MJNC, Struijk PC, Wladimiroff JW, Steegers EAP (Rotterdam, The Netherlands): Longitudinal determination of placental vascularization index during the second half of pregnancy, using 3D power Doppler ultrasound; first results of a pilot study
- 109.** Hajdú J, Beke A, Marton T, Hruby E, Pete B, Papp Z (Budapest, Hungary): Congenital heart diseases in twin pregnancies
- 110.** Harmath Á (Budapest, Hungary): Congenital diaphragmatic hernia: Changing the patient's admission to genetic counselling on the basis of 24 years data
- 111.** Hodik K, Musilova I, Natekova J, Elias P, Podholova M (Hradec Kralove, Czech Republic): Intrauterine ultrasound guided laser fotocoagulation for acardiac twin
- 112.** Horovitz J, Deckindt C, Mangione R, Guyon F, Saura R (Bordeaux, France): Isolated fetal ascites detected by sonography
- 113.** Hruby E (Budapest, Hungary): Twin pregnancies complicated by intrauterine death of one co-twin: maternal risks of expectative management
- 114.** Joó JG, Beke A, Papp Cs, Tóth-Pál E, Szigeti Zs, Bán Z, Papp Z (Budapest, Hungary): Prenatal diagnosis, phenotypic and obstetric characteristics of holoprosencephaly
- 115.** Katsoulis I, Papageorgiou I, Papantoniou N, Antsaklis A (Athens, Greece): Hereditary long QT syndrome in pregnancy. Antenatal and intrapartum management options
- 116.** Kovács Z, Rigó J Jr. (Budapest, Hungary): Fetal consequences of opiate use in pregnancy
- 117.** Krasznai I, Szendei G, Garamvölgyi Z, Dévényi N, Bóze T, Rigó J (Budapest, Hungary): Androgens as markers of preeclampsia
- 118.** Nagy S, Bush M, Lapinski R, Gardó S (Győr, Hungary, New York, USA): Clinical significance of subchorionic and retroplacental haematomas detected in the first trimester of pregnancy
- 119.** Pajkrt E, Chitty L (London, UK): Prenatal diagnosis of joint contractures: karyotype, associated findings and outcome
- 120.** Papitashvili A (Tbilisi, Georgia): First trimester ultrasonography screening for fetal abnormalities: 24 years of studies
- 121.** Pete B, Hajdú J, Papp Z (Budapest, Hungary): Diagnosis and treatment of haemodynamically significant fetal tachycardia - review of 33 cases
- 122.** Shulman LP (Chicago, USA): Invasive prenatal diagnostic procedure decision-making in women undergoing multifetal pregnancy reduction
- 123.** Than N, Magenheimer R, Boronkai Á, Deres P, Bellyei Sz, Hargitai B, Szigeti A, Rigó J Jr, Sümegi B, Papp Z (Budapest and Pécs, Hungary): Placental origin of the extreme elevation of maternal serum ALP levels
- 124.** Theodora M, Papageorgiou J, Daskalakis G, Antsaklis A (Athens, Greece): Hypertensive disease and

evolution of pregnancy. A retrospective study

**125.** Theodora M, Papageorgiou J, Daskalakis G, Antsaklis A (Athens, Greece): Prelabor rupture of membranes and evolution of pregnancy. A retrospective study

**126.** Theodora M, Papageorgiou J, Daskalakis G, Antsaklis A (Athens, Greece): Diabetes mellitus and evolution of pregnancy - A retrospective study

**127.** Wojakovski A, Otano L, Elías D, Dovasio F, Izbizky G, Aiello H, Farias P (Buenos Aires, Argentina): Fetal dacrocystocel: 3 cases with spontaneous prenatal resolution

## Sunday, 27 June

---

### 11.00-14.00 Societal aspects of prenatal diagnosis

Chair: **Sciarra J (Chicago, USA)** and **Harer B (San Bernardino, USA)**

---

#### Oral presentations

**128.** Carrera JM (Barcelona, Spain): The situation of prenatal diagnosis in different continents

**129.** Holzgreve W (Basel, Switzerland): First trimester biochemical and ultrasound marker screening - practical aspects

**130.** Prudent L (Buenos Aires, Argentina): Maternal-fetal conflicts. A pediatrician perspective

**131.** Gadow EC, Krupitzki H (Buenos Aires, Argentina): Awareness and attitude toward prenatal diagnosis in countries without legal termination of pregnancy

#### Coffee Break

**132.** Marteau T, Dormandy E (London, UK): Informed choice: Bridging the gap between policy and practice

**133.** Quadrelli R, Vaglio A (Montevideo, Uruguay): Parental decision to abort or continue a pregnancy with cytogenetic abnormal finding after an invasive prenatal test

**134.** Nakano H (Fukuoka, Japan): Fetal learning

**135.** Milunsky A (Boston, USA): Clinical genetics and maternal-fetal medicine: Lessons from the law

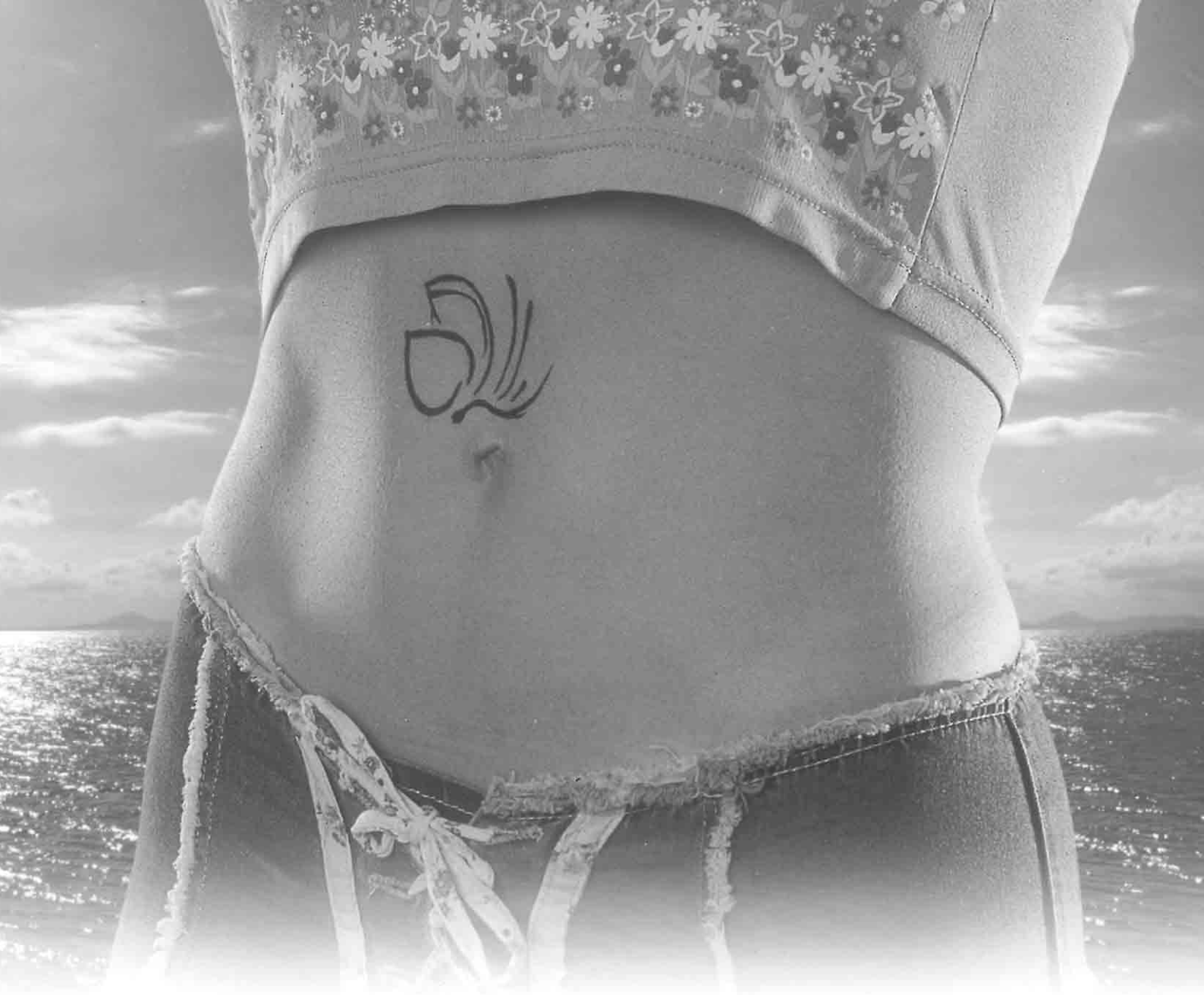
**136.** Papp Z (Budapest, Hungary): Change in public demand for genetic counseling in the past 30 years

## 14.00 Closing

---

**Rodeck, Charles (London, UK)** – President of the ISPD

**Papp, Zoltán (Budapest, Hungary)** – President of the Conference



**Lindynette<sup>®</sup>**

*Butterfly light freedom*

*ethinylestradiol, gestodene*

*The newest very-low dose 3<sup>rd</sup>  
generation oral contraceptives from...*



**RICHTER GEDEON LTD.**

---

Marketing Department of Gynaecological Products  
H-1103 Budapest, Gyömrői út 44.  
Phone: 36-1-431-5314, Fax: 36-1-431-4449

## ABSTRACTS

---

## 1. PREIMPLANTATION GENETIC DIAGNOSIS

**J.L. Simpson**

Department of Obstetrics and Gynecology, Baylor College of Medicine, Houston, Texas, USA

Preimplantation genetic diagnosis (PGD) is an integral part of the prenatal diagnosis armamentarium, complementary to traditional prenatal genetic diagnosis (PND). PGD is selected by some couples as an option vis a vis traditional prenatal genetic diagnosis (chorionic villus sampling or amniocentesis), whereas others chose PGD because their needs are not met by traditional approaches.

1) PGD vs PND -Initially preimplantation genetic diagnosis was performed only for high-risk circumstances, such as Mendelian disorders. Emphasis was on conditions in which there was a high absolute risk. Using FISH with chromosome-specific probes resulted in the option of cytogenetic diagnosis, but again prediction was given to high-risk situations like reciprocal translocations. However, it became clear that many couples were choosing PGD not solely because of high-risk but to avoid clinical termination of pregnancy.

2) Aneuloidy screening soon became widely utilized to improve pregnancy rates in IVF failures, repeated spontaneous abortions and simply maternal age. Some women were of advanced maternal age, and were undergoing IVF to achieve pregnancies. With transfer of euploid embryos, clinical abortion rates are decreased; selecting patients who benefit with increased live born babies has proved more difficult.

3) PGD for non-disclosure is one novel application, which arose as the public urged the scientific community to apply the new technology beyond traditional indications. This indication applies to PGD adult onset disorders like Huntington disease.

4) PGD or selection of HLA-compatible embryos -If an older sib is moribund because of a bone marrow infiltrative disease (e.g., Fanconi anemia), the couple may wish to have an unaffected younger sib. If so, one could harvest that infant's cord blood and, if HLA-compatible use for stem cell therapy in its older sib.

Conclusion: Preimplantation genetic diagnosis (PGD) has evolved from a strictly narrow genetic focus to broader, novel, indications not initially envisioned by the genetic community. The public will continue to expect newer applications, well beyond eschewing birth of an infant with a lethal childhood-onset disorder.

## 2. ORIGIN OF ANEUPLOIDIES IN PREIMPLANTATION EMBRYOS

**A. Kuliev, J. Cieslak, Z. Zlatopolsky, Y. Illkevitch, I. Kirilova, Y. Verlinsky**

Reproductive Genetics Institute, Chicago, Illinois, USA

OBJECTIVE: The majority of chromosomal abnormalities originate from female meiosis, but the mechanism of errors and the stages of meiosis at which these errors are originated are not sufficiently understood.

DESIGN AND METHODS: We studied more than 10,000 oocytes obtained in IVF cycles from patients of advanced maternal age, using fluorescent in situ hybridization (FISH) analysis of the outcome of meiosis I and meiosis II, with application of commercial probes specific for five chromosomes (chromosomes 13,16, 18, 21 and 22).

RESULTS: Over 53% of oocytes with available FISH results were with aneuploidies, which is in the same range as chromosomal abnormalities in cleaving embryos detected in PGD of aneuploidies at the cleavage stage. 39% of errors in oocytes originated from meiosis I, 32% from meiosis II, and the remaining 31% from both meiosis I and II. The majority of meiosis I errors were chromatid malsegregations, which were observed in 27% of all oocytes, including 20% resulting in extra chromatid MII oocytes and 7% with missing chromatids, while there were no differences for extra and missing chromatids in oocytes following meiosis II. At least one third of sequential errors in meiosis I and II, resulting in a balanced karyotype of the resulting zygotes, however the majority of them developing in the chromosomally abnormal embryos following postzygotic embryonic development, half of which were with mosaicism, involving the same or different chromosomes.

CONCLUSIONS: The data suggest the requirement for oocyte testing for accurate assessment of chromosomal status of the resulting embryos, as chromosomal errors in meiosis may be a possible indicator of further chromosomal errors at the cleavage stage.

### **3. PREIMPLANTATION DIAGNOSIS FOR SINGLE GENE DEFECTS AND ITS APPLICATION TO PREIMPLANTATION HLA TYPING**

**Y. Verlinsky, S. Rechitsky, A. Kuliev**

Reproductive Genetics Institute, Chicago, Illinois, USA

**OBJECTIVE:** More than 1500 preimplantation genetic diagnosis (PGD) cycles have been performed for single gene disorders and dynamic mutations, resulting in more than 300 unaffected children born by the present time. The available experience shows application of PGD to those conditions that have never represented indication for prenatal diagnosis.

**DESIGN AND METHODS:** Our experience involves approximately 500 PGD cycles performed for 98 different conditions, including single gene defects, dynamic mutations and some medically relevant genetic variations. An improved multiplex PCR protocols were developed, involving simultaneous testing for the causative mutation together with at least three linked markers, aneuploidy and non-disease variations, such as pre-selection of the HLA compatible donors for affected siblings.

**RESULTS:** Unaffected embryos for transfer were pre-selected in 89% of clinical cycles, resulting in clinical unaffected pregnancies in 35% of cases, resulting in birth of more than one hundred healthy children. This included HLA genotyping in two dozen cycles in combination with PGD for thalassemia, Fanconi anemia, hyper immunoglobulin M syndrome, X-linked adrenoleukodystrophy and Wiscott-Aldrich syndrome, more than a dozen cycles for preimplantation HLA typing without testing for causative gene, such as for different leukemias, and Diamond-Blackfan anemia. This resulted in the embryo transfer of HLA matched embryos in almost all cycles, yielding clinical pregnancies and births of HLA-matched healthy children to become potential HLA compatible donors for their siblings requiring bone marrow transplantation.

**CONCLUSIONS:** PGD is currently expanded for wider indications than those practiced in prenatal diagnosis. These new indications already represent a significant proportion of PGD experience, with only HLA cases approaching 7% of our experience for Mendelian disorders, making PGD an important complement to prenatal diagnosis.

### **4. THE VALUE OF PREIMPLANTATION GENETIC DIAGNOSIS AS A CLINICAL PROGNOSTIC TOOL**

**L. Gianaroli, M.C. Magli, A.P. Ferraretti**

S.I.S.Me.R., Reproductive Medicine Unit, Bologna, ITALY

**OBJECTIVE:** To evaluate the results obtained from the chromosomal analysis of preimplantation embryos in terms of prediction of embryo viability.

**DESIGN AND METHODS:** Patients with a poor prognosis for pregnancy underwent assisted conception cycles in combination with PGD for aneuploidy. The numerical analysis of chromosomes in interphase nuclei was performed with the in situ hybridization technique (FISH) using probes specific for the chromosomes XY, 13, 15, 16, 18, 21 and 22.

**RESULTS:** Approximately 33% of the 5102 FISH diagnosed embryos were classified as chromosomally normal. The analysis of the results obtained revealed a correlation between chromosomal status and 1) pronuclear zygote morphology, 2) embryo morphology and cleavage rate, and 3) blastocyst development. Furthermore, the retrospective analysis of the results derived from patients who repeated at least twice a cycle with PGD for aneuploidy demonstrated that the chances of on-term pregnancy in subsequent cycles were below 10% when no euploid embryos were detected at the first attempt and were approximately 30% for couples with at least two euploid embryos in the first cycle.

**CONCLUSIONS:** Patients with a poor prognosis of pregnancy have a tendency to reproduce the same pattern of chromosomal abnormalities through subsequent cycles. Consequently, the performance at their first cycle represents a prognostic tool for the evaluation of their chances of pregnancy in subsequent attempts.

## **5. PGD TO REDUCE SPONTANEOUS ABORTIONS IN TRANSLOCATION CARRIERS AND PATIENTS WITH RECURRENT MISCARRIAGES**

**S. Munne<sup>1,2</sup>, T. Escudero<sup>3</sup>, J. Fischer<sup>2</sup>, P. Colls<sup>2</sup>, X. Zheng<sup>2</sup>, O. Maria<sup>3</sup>, J. Cohen<sup>1,2</sup>**

<sup>1</sup>Yale University, New Haven, CT, <sup>2</sup>Reprogenetics, West Orange, NJ, <sup>3</sup>Reprogenetics, South San Francisco, CA, USA

Preimplantation Genetic Diagnosis for chromosome abnormalities has been applied to carriers of translocations and inversions, as well as for the indications of advanced maternal age, repeated implantation failure, and other indications. Some studies have shown an improvement of implantation, pregnancy rates, and/or take-home baby rates and a reduction in spontaneous abortions (Munné et al. 1999, 2003; Gianaroli et al. 1999).

Two groups of patients are at much higher risk pregnancy loss, one being carriers of translocations, and the other none translocation carriers with different etiologies but 50% of them idiopathic. For both groups high rates of chromosomally abnormal embryos have been reported (Munne et al. 1998, 2000; Pellicer et al. 1999, Rubio et al. 2003).

Results from PGD cycles referred to our reference lab from over 80 IVF centers indicate that PGD is a very successful tool in reducing spontaneous abortions, from an 84% (n=278) miscarriage rate before PGD, to 5% (n=78) after PGD. None of the babies born or karyotyped abortuses were affected. The pregnancy rate was correlated with the number of abnormal embryos. Couples with more than 70% embryos abnormal for the translocation seldom become pregnant, probably because in addition there is a baseline of 30-60% chromosome abnormalities not related to the translocation.

A group of recurrent miscarries (3 or more losses) of idiopathic etiology underwent PGD for aneuploidy. They had previously miscarried 87% of their pregnancies (n=301). According to a formula by Brigham et al (1999) and their previous losses and maternal age, a 36% pregnancy loss in the next cycle would be expected but only 17% occurred (p=0.028). In the RM patients 35 and older, the expected rate of miscarriage would had been 45% and it was only 12% after PGD (p=0.007). Thus, PGD of aneuploidy seems an effective procedure for treating idiopathic recurrent pregnancy loss.

## **6. INCREASING EFFICACY AND SAFETY OF PREIMPLANTATION GENETIC DIAGNOSIS FOR MONOGENIC DISEASES. ONE-CELL VERSUS TWO-CELL BIOPSY: A DIFFICULT CHOICE**

**K. Sermon**

Centre for Medical Genetics, University Hospital and Medical School of the Brussels Free University, Brussels, BELGIUM

**OBJECTIVE:** Because of the occurrence of misdiagnoses after PGD for monogenic diseases, the search for more accurate and efficient methods for single cell PCR has been a constant priority. These include the introduction of fluorescent PCR and multiplex PCR combining PCR for the causative mutation(s) together with informative markers, or a combination of several of these markers. Newer technologies, which are already widely used for routine (prenatal) genetic diagnosis, are continuously downscaled to the single cell level. Examples are minisequencing, automated sequencing, real-time PCR and the use of microarrays. An overview of these new technologies and how they apply to single cell PCR will be given. The last possibility to increase accuracy is to biopsy two cells from an embryo. However, this has met with concerns about the safety and survival of the embryo and the possible pregnancy and child ensuing from this embryo.

**DESIGN AND METHODS:** We are currently conducting a study comparing one-cell and two-cell biopsy in a group of PGDs for monogenic diseases where duplex or multiplex PCR can be applied. The parameters followed are: efficiency (no diagnosis) and accuracy (misdiagnosis) of diagnosis, further embryo development, and implantation rate and ongoing pregnancy rates. As of January 2004, there were 34 cycles in the one-cell group with 212 embryos analysed and 36 cycles in the two-cell group, with 215 embryos analysed.

**RESULTS:** The number of embryos without diagnosis was 32 in the one-cell group (15.1 %) and 11 in the two-cell group (5.1 %). No misdiagnoses occurred in either group. Because the groups are too small, no conclusions can be drawn as to the implantation rates and ongoing pregnancy rates.

**CONCLUSIONS:** We conclude that modern technology renders PGD nearly as safe and accurate as prenatal diagnosis.

## 7. MICROARRAYS FOR ANALYSIS AND DIAGNOSIS OF PREIMPLANTATION EMBRYOS

**D. Wells<sup>1</sup>, M. Bermudez<sup>2</sup>, N. Steuerwald<sup>2,4</sup>, L. Chu<sup>3</sup>, U. Weier<sup>3</sup>, J. Cohen<sup>4</sup>, S. Munne<sup>2,4</sup>**

<sup>1</sup>ART Inst. of NY & NJ, West Orange, NJ; <sup>2</sup>Reprogenetics, Hoboken, NJ; <sup>3</sup>University of California, Lawrence Berkeley National Laboratory, CA; <sup>4</sup>Tyho-Galileo Research Laboratories, West Orange, NJ, USA

**OBJECTIVE:** To develop methods permitting the analysis of DNA sequence, gene expression and aneuploidy in minute quantities of tissue (e.g. single cells) using microarrays.

**DESIGN AND METHODS:** We utilized commercially available microarrays for the investigation of gene expression and for identifying differences in DNA sequence. For cytogenetic analysis we employed a microarray of our own design. Central to each form of analysis was the method used for amplifying nucleic acids. Amplification of DNA was accomplished using degenerate oligonucleotide primed PCR (DOP-PCR), while amplification of RNA involved the use of an *in vitro* transcription reaction.

**RESULTS:** DOP-PCR produced sufficient DNA from single cells for aneuploidy screening using our custom-microarray. This permitted simultaneous evaluation of 18 chromosomes, including those most often involved in prenatal aneuploidy. Aneuploidy was detected in cells from 3/3 cell-lines containing chromosome imbalance. DNA sequence was analyzed using an Affymetrix microarray that has the potential to simultaneously genotype 10,000 single nucleotide polymorphisms, providing information on disease inheritance via linkage analysis and in some cases ploidy. This method is still under development, but has already allowed genotyping of several thousand SNPs in single cells. Genome Focus Arrays (Affymetrix) were employed to assess gene expression in single cells. These microarrays provide data on ~8,500 genes and were successfully applied to single oocytes.

**CONCLUSIONS:** This study demonstrates that microarrays can be employed for single cell analysis. Application of this technology for preimplantation genetic testing could allow comprehensive aneuploidy screening and simplify approaches for the detection of disease causing mutations. Additionally, simultaneous analysis of mRNA from thousands of genes in individual oocytes/embryos will add much to our understanding of the biochemical pathways active at different stages of development, potentially leading to improved IVF and embryo culture.

## 8. FUTURE DEVELOPMENTS IN PREIMPLANTATION GENETIC DIAGNOSIS

**J. Harper**

UCL Centre for Preimplantation Genetic Diagnosis, University College London, UK

PGD is a continually evolving field. However, the biopsy technique has remained relatively unchanged, except with the recent introduction of using a laser for zona drilling. Most developments concern the diagnosis.

FISH is limited by the number of probes that can be used at one time. Reprobing methods have allowed more probes to be used, but still not all chromosomes can be examined. The development of techniques that allow analysis of all chromosomes is essential for PGS and would make PGD for chromosome abnormalities more routine. Comparative genomic hybridisation (CGH) is such a technique, but it is time consuming and technically difficult.

Single cell PCR has seen many improvements since the start of PGD. Most groups now use fluorescent PCR with linked or unlinked markers for contamination detection. The use of microarrays may make such diagnosis simpler to develop.

Guidelines for PGD have been written by both the PGDIS and the ESHRE PGD Consortium. Since PGD is such a technically challenging procedure, these guidelines will assist centres setting up PGD.

## 9. A CONTROLLED STUDY FOR GENDER SELECTION USING SWIM-UP SEPARATION

**M. Khatamee, S. Horn, A. Weseley, T. Farooq, S. Jaffe, R Jewelwicz**

NYU School of Medicine, Fertility Research Foundation, New York, USA

**OBJECTIVE:** To evaluate the success for gender selection using a sample of semen separated by a modified swim-up technique.

**DESIGN AND METHODS:** We retrospectively compared the gender outcome of two treatments (A and B) for either a male or female offspring with those who conceived spontaneously. *Setting:* Private practice of one author (M.K). *Patients, Participants:* The treatment groups consisted of 52 total pregnancies for couples who conceived by the separation technique. Of these 52 participants, 15 desired a female offspring and were placed into treatment A and 37 desired a male offspring and were placed into treatment B. The control groups consisted of 162 women who were presented with initial consultation for gender selection and conceived spontaneously. Control group A consisted of 80 women who initially chose a female offspring, and control group B consisted of 82 participants who initially chose a male. *Interventions:* In treatment group A, one timed intrauterine insemination (IUI) was curled out with the bottom 0.5 ml of the separated semen on cycle days 12-14, when the follicle was 18-22 mm. Patients in this group were also administered clomiphene citrate and human chorionic gonadotropin, in treatment group B, one timed IUI was done with the top 0.5 ml of the semen, when the follicle was 18-22 mm. *Main Outcome Measure:* The gender outcome of the pregnancies of two treatment and control groups was evaluated based on the known desired gender.

**RESULTS:** The success rate for conceiving a female child after intervention (treatment group A) was 86.7 % effective ( $p=0.002$ ) as compared to the control group A. Couples seeking a male child (treatment group B) were 89.2% effective ( $p=0.0002$ ) as compared to the control group B.

**CONCLUSIONS:** This study reveals that the modified swim-up method with additional monitoring results in statistically significant gender pre-selection.

## 10. DETAILED FISH ANALYSIS ON DAY 5 HUMAN EMBRYOS REVEALS THE MECHANISMS LEADING TO MOSAIC

**D. Daphnis<sup>1</sup>, J. Harper<sup>1</sup>, S. Jerkovic<sup>2</sup>, J. Geyer<sup>2</sup>, I. Craft<sup>2</sup>, J. Delhanty<sup>1</sup>**

<sup>1</sup>UCL Centre for PGD, Department of Obstetrics and Gynaecology, University College London, UK

<sup>2</sup>London Fertility Centre, London, UK

**OBJECTIVE:** The aim of this study was to determine the true level of mosaicism by excluding FISH artefacts and to obtain information concerning the mechanisms responsible for generating aneuploidy mosaicism.

**DESIGN AND METHODS:** Embryos were cultured in different types of IVF medium. Group I consisted of embryos that were cultured in standard IVF medium and Group II consisted of embryos cultured from day 3-5 in blastocyst medium. Embryos were spread using HCl and Tween 20 and three rounds of FISH were performed. In round 1 the probes used were 1p (Spectrum Green), 11q (Spectrum Orange) and 18CEP (Spectrum Aqua); in round 2 the probes used were 1satII/III (Spectrum Aqua), 11CEP (Spectrum Green) and 18q (Spectrum Orange). And in round 3 the probes used were 18CEP(Spectrum Aqua), XCEP (Spectrum Green) and YCEP (Spectrum Orange).

**RESULTS:** A total of 21 embryos were analysed in each Group. Group I embryos had significantly less cells than Group II embryos (Group I,  $n=410$  and Group II,  $n=1171$ ). The FISH results revealed 1 uniformly diploid and 20 mosaic embryos for Group I and 2 uniformly diploid and 19 mosaic embryos for Group II. However, both Group I and II showed a high percentage of diploid cells (73% and 78% respectively).

**CONCLUSIONS:** FISH has been used for the study of chromosomes in embryos for over 12 years. Results have shown that human embryos display a high level of chromosome abnormalities at all preimplantation stages. Mosaicism is the most common abnormality observed. The use of 2 different probes per chromosome was able to detect FISH artefacts and failure of hybridisation, which was 6.5% (26/410) for Group I and 4.5% (50/1171) for Group II. The relatively high percentage (7%) of tetraploidy in Group II was considered to reflect normal embryonic development. Post-zygotic chromosome loss was the predominant mechanism leading to aneuploidy mosaicism for both groups, followed by chromosome gain, with some examples of mitotic non-disjunction.

## 11. MOLECULAR CYTOGENETIC INVESTIGATIONS OF ANEUPLOIDY: FISH AND CGH ANALYSIS OF HUMAN OOCYTES AND POLAR BODIES

**E. Fragouli<sup>1</sup>, C.M. Conn<sup>1</sup>, S. Cupisti<sup>5</sup>, D. Wells<sup>4</sup>, K. Whaley<sup>2</sup>, J.A. Mills<sup>2</sup>, M.J.W. Faed<sup>3</sup>, J.D.A. Delhanty<sup>1</sup>**

<sup>1</sup>Department of Obstetrics and Gynecology, University College London, UK, <sup>2</sup>Assisted Conception Unit, Tayside University Hospitals Trust, <sup>3</sup>Department of Cellular and Molecular Pathology, University of Dundee, Ninewells Hospital, Dundee, UK, <sup>4</sup>The Institute of Reproductive Medicine and Science, St Barnabas Medical Center, New Jersey, USA, <sup>5</sup>Friedrich Alexander-Universitat, Erlangen-Nurnberg, Frauenklinik, Erlangen, GERMANY

**OBJECTIVE:** Aneuploidy is an order of magnitude greater in humans compared to other mammals, and leads to high rates of mental retardation and pregnancy wastage. It has been demonstrated that errors taking place during the first meiotic division in females are the main cause of numerical abnormalities. The mechanisms proposed are two: firstly the malsegregation of whole univalent chromosomes, secondly the predivision of sister chromatids prior to meiotic anaphase I. Smaller autosome aneuploidies have been particularly associated with advancing maternal age, while absent or aberrant genetic recombination was identified as a generally predisposing factor. The aim of our study was the investigation of mechanisms leading to aneuploidy of specific chromosomes in younger IVF patients.

**DESIGN AND METHODS:** Human oocytes and associated 1<sup>st</sup> polar bodies (PBs) were analysed where possible. Most of these had failed to fertilise after exposure to sperm. In the first part of the study, fluorescence *in situ* hybridisation (FISH) was performed in three sequential rounds, using probes for chromosomes 1, 9, 12, 13, 16, 18, 21 and X. Only imbalances due to gain of a whole chromosome or chromatid were counted to avoid artefactual scoring of chromosome loss. During the second part, oocytes and 1<sup>st</sup> PBs were analysed by applying Comparative Genomic Hybridisation. As CGH is a DNA based technique, it is able to investigate the whole maternal genome and thus provide information on chromosome copy number and hypohaploidy status.

**RESULTS:** FISH data was available on 244 eggs from 129 patients of average age 32.5 years (range 22-44). Ten patients (average age 32.6) showed chromosome abnormalities in their eggs, the rate being 4%. Hyperhaploidies were observed in chromosomes 13, 16, 18, 21 and X, but not 1, 9 or 12. CGH yielded results on eleven unfertilised oocytes and 15 PBs to date. Abnormalities were identified for two oocytes and five PBs.

**CONCLUSIONS:** The study of this patient group revealed several mechanisms leading to aneuploidy. Included are: firstly, the classical whole univalent non-disjunction, secondly, the precocious separation of chromatids prior to anaphase I, leading to imbalance detected at metaphase II, and thirdly, the presence of gonadal or germinal mosaicism for a trisomic cell line. Evidence for the preferential involvement of smaller chromosomes was also provided. These observations led to the conclusion that mechanisms causing human aneuploidy are chromosome-specific and some may be age-independent.

## 12. EXPRESSION OF PLATELET-ACTIVATING FACTOR ACETYLDHROLASE (Ib) AND PAF-RECEPTOR IN THE HUMAN OOCYTE

**A.L. Garda<sup>1,2</sup>, S. Martínez<sup>2</sup>, E. Gómez<sup>1</sup>, M.C. Martínez<sup>1</sup>, I. Pérez<sup>1</sup>, B. Amorcho<sup>1</sup>, J. Landeras<sup>1</sup>, A. Ballesteros<sup>1</sup>**

<sup>1</sup>Molecular Genetics Unit, Instituto Valenciano de Infertilidad, Murcia, SPAIN

<sup>2</sup>Instituto de Neurociencias, CSIC and Miguel Hernandez University, San Juan, SPAIN

**OBJECTIVE:** The General aim of our project is to investigate the effect of the molecules associated with the platelet activating factor in human implantation. We have analysed the expression of the subunits of platelet activating factor acetylhydrolase Ib (PAF-AH) and PAF-Receptor in the human oocyte, in order to relate them with the earliest reproductive events. **DESIGN AND METHODS:** Oocytes were obtained after the consent of donors who received hormonal stimulation and participating in a program of assisted reproduction. Each oocyte was separated from the associated cumulus cells by enzymatic treatment. Immediately after the dissociation of cumulus cells, the oocyte was washed with culture medium, twice in PBS and transferred in a minimal volume of PBS into TRIZOL-Reagent, containing glycerol as a carrier. RNA extraction and RT-PCR reactions were performed using conventional protocols. The analysis of the PAF-Receptor and PAF-AH (Ib) was made by the nested PCRs amplifications of the 3' regions cDNA sequences. In the former the primers were designed for its LIS1, a1 and a2 subunits. All amplifications were made in presence of positive and negative controls.

**RESULTS:** The presence of PAF-AH(Ib) subunits in oocytes was investigate in oocytes (n=40) using RT-PCR. We were able to detect mRNA expression of all three PAF-AH (Ib) subunits with the expected molecular size. We suspect that both of them are expressed at protein level as was demonstrated by Cahana et al (FEBS Lett, 1999; 451:99) in mouse. Otherwise we did not observed the mRNA of PAF-R in the PCR reactions suggesting that this receptor is not expressed in the oocyte and is not present in it during the implantation process.

**CONCLUSIONS:** This is the first report of maternal human expression of the PAF-AH (Ib) subunits at one oocyte stage. Further quantitative analysis for these subunits at cellular level may allow us to determine in the future about the diagnostic possibilities of this molecular model in the oocyte quality and early reproductive impairment.

## 13. PREIMPLANTATION GENETIC DIAGNOSIS FOR ASHKENAZI JEWISH GENETIC DISORDERS

**M. Malcov, Z. Frumkin, T. Schwartz, N. Mey-Raz, A. Amit, D. Ben Yosef, F. Azem, J.B. Lessing, Y. Yaron**

IVF Unit, Lis Maternity Hospital and Prenatal Diagnosis Unit, Genetic Institute, Sourasky Medical Center, Tel Aviv, ISRAEL

**OBJECTIVE:** The Ashkenazi Jewish population in Israel is characterized by a number of relatively common genetic disorders. The purpose of this study is to develop PGD protocols for the common disorders among Ashkenazi Jews: cystic fibrosis (CF), familial dysautonomia (FD), Canavan disease, Bloom syndrome and fragile X.

**DESIGN AND METHODS:** The protocols were first developed on single leukocytes obtained from normal individuals, carriers, and affected individuals. For Canavan disease, we developed duplex PCR method to analyze for the 2 common *ASPA gene* mutations. For Bloom syndrome and familial dysautonomia we developed single cell nested PCR protocols for the single common mutation in each disorder. For cystic fibrosis we develop different PCR protocols for the different common *CFTR* mutations. For the fragile X syndrome, caused by triplet CGG expansion, we developed several duplex nested PCR sets, for the simultaneous amplification of the CGG triplet repeat region in the FMR1 gene, as well as one of the adjacent polymorphic markers. The sensitivity of the tests in single leukocytes and single blastomeres exceeded 95%.

**RESULTS:** We performed 2 PGD cycles for Canavan disease and have one ongoing pregnancy, confirmed to be unaffected by amniocentesis. For Bloom syndrome we performed 2 PGD cycles, but the patient proved to be a low responder. For familial dysautonomia we performed 3 PGD cycles but no pregnancy was yet achieved. For cystic fibrosis we performed one PGD cycle, but no pregnancy was obtained. We are currently offering PGD for fragile X to appropriate candidates.

**CONCLUSIONS:** Reliable PGD for the common Ashkenazi Jewish disorders offers for carrier couples an alternative to prenatal diagnosis and termination of affected pregnancies. This is particularly important among Orthodox Jews, individuals opposed to termination of pregnancy or to patients undergoing in vitro fertilization.

#### 14. PREIMPLANTATION GENETIC DIAGNOSIS EXPERIENCE FOR SINGLE-GENE DISEASES: INITIAL RESULTS

**J. Martín<sup>1</sup>, C. Rubio<sup>1</sup>, A. Mercader<sup>1</sup>, C. Simón<sup>1,2</sup>, J. Remohí<sup>1,2</sup>, A. Pellicer<sup>1,2</sup>**

<sup>1</sup>Instituto Valenciano de Infertilidad (IVI group), <sup>2</sup>Department of Obstetrics and Gynecology, School of Medicine, University of Valencia, SPAIN

**OBJECTIVE:** Our purpose was to show our initial experience in the diagnosis of monogenic diseases in a preimplantation genetic diagnosis (PGD) program.

**DESIGN AND METHODS:** Day 3-embryos were biopsied using tyrode's acid (first cases) or laser (currently) to retrieve preferentially two blastomeres. One blastomere could be obtained for slow embryos whenever the disease's diagnosis was based on the analysis of two markers (STRs). Fluorescent PCR and automated genetic analysis (using ABI PRISM™ 310 Genetic Analyzer, Applied Biosystem) was the choice to assess the embryo's genetic status. Working protocols were adapted from the PGD group in the Centre for Medical Genetics (AZ-VUB, Brussels). In total, 14 PGD cycles were performed for 7 monogenic diseases: hemophilia A (4 cases), spinal muscular atrophy (SMA) (3), fragile X syndrome (2), autosomal dominant polycystic kidney disease (2), cystic fibrosis (1), myotonic dystrophy (Steinert's disease) (1) and X-linked Alport syndrome (1).

**RESULTS:** Since November 2002, sixteen couples have been treated in our clinic with indication of PGD for monogenic diseases. In total, 18 cycles were started; of these, diagnosis was performed for 14 cycles (78%). Transfer was available for 10 cycles (70%). Our pregnancy rate was 60%. After ultrasonographic control, one unembryonic pregnancy was interrupted (17%). In total, three healthy babies have been born (one single pregnancy for SMA and one twin-pregnancy for hemophilia); three pregnancies are currently ongoing.

**CONCLUSIONS:** Our results corroborate that PGD for monogenic diseases can be offered as an efficient option for couples at risk of having affected offspring.

#### 15. FISH ANALYSIS IN SPERM SAMPLES FROM PATIENTS WITH RECURRENT HYDATIDIFORM MOLES

**E. Mateu, C. Rubio, L. Rodrigo, C. Serrano, J. Remohí, A. Pellicer**

Instituto Valenciano de Infertilidad, Valencia, SPAIN

**OBJECTIVE:** Partial and complete hydatidiform mole are chromosomally abnormal pregnancies due to triploidy or to diploidy, with the two sets of chromosomes belonging to the father. The objective of this study is to analyse the incidence of diploid and aneuploid spermatozoa in patients with recurrent hydatidiform moles.

**DESIGN AND METHODS:** This study includes three sperm samples from patients with normal karyotypes and two previous molar pregnancies each one. Triple FISH was performed for chromosomes X, Y and 18, and dual FISH for chromosomes 13 and 21 (Vysis Inc. Downers Grove, IL, USA). Chi-square test was applied to compare the incidence of disomy and diploidy with a control group of normozoospermic donors.

**RESULTS:** One of the patients showed a significant increase in the percentage of diploid spermatozoa compared to the control group (0.72% vs 0.25%;  $p < 0.0001$ ). No significant differences in the percentage of chromosomal abnormalities for the chromosomes analysed were found in the other two patients compared to the control group.

**CONCLUSIONS:** FISH analysis in spermatozoa allowed us to identify an increased incidence of diploid spermatozoa in one of the patients. We suggest that diploid sperm could be related with the origin of the recurrent hydatidiform mole in some cases.

## 16. PGD IN ADVANCED MATERNAL AGE: INCIDENCE OF CHROMOSOMAL ABNORMALITIES AND PREGNANCY OUTCOME

**I. Pérez<sup>1</sup>, C. Rubio<sup>2</sup>, L. Rodrigo<sup>2</sup>, A. Mercader<sup>2</sup>, E. Mateu<sup>2</sup>, A. Ballesteros<sup>1</sup>, J. Landeras<sup>1</sup>, J. Remohí<sup>2,3</sup>, A. Pellicer<sup>2,3</sup>**

<sup>1</sup>Instituto Valenciano de Infertilidad IVI-Murcia, Murcia, <sup>2</sup>Instituto Valenciano de Infertilidad IVI-Valencia, Valencia,

<sup>3</sup>Department of Obstetrics and Gynecology, School of Medicine, University of Valencia, Valencia, SPAIN

**OBJECTIVE:** Aneuploidies and spontaneous abortions increase with maternal age. The objective of the present study was to evaluate the incidence of chromosomal abnormalities as well as pregnancy outcome in women  $\geq 38$  years of age.

**DESIGN AND METHODS:** Aneuploidy screening due to advanced maternal age was performed in 335 cycles with maternal age  $\geq 38$  years. Embryo biopsy was performed on day 3 and chromosomes 13, 16, 18, 21, 22, X and Y were analysed in two/three hybridization rounds (Vysis Inc., Downers Grove, IL). On day 5, chromosomally normal embryos were transferred either at morula or blastocyst stage. The incidence of chromosomal abnormalities, pregnancy, implantation and miscarriage rates were evaluated in five different groups according to maternal age: 38 years, 39 years, 40 years, 41 years and  $\geq 42$  years.

**RESULTS:** In total, 1313 day 3 embryos were analysed and the percentage of chromosomal abnormalities was 72.5%. The incidence of abnormalities increased from 60% in women of 38 years to 80.7% in women  $\geq 42$  years, mainly due to chromosome 21 and sex chromosomes. In patients of 38 years of age chromosomally normal embryos were available for transfer in 84.6% of the cycles and only in 34 % of the patients over 41 years of age. Pregnancy rates for patients up to 41 years of age, ranged from 45.5% to 21%, with only 6.3% in women  $\geq 42$  years. Implantation rates followed a similar pattern whereas miscarriage rates were low, with a mean value of 8.7%.

**CONCLUSIONS:** PGD to discard aneuploidies can be successfully applied in advanced maternal age until the age of 41 years. Women over 41 years of age displayed extremely high incidence of chromosomal abnormalities with very low implantation rates, indicating that other alternatives such as ovum donation should be considered in this age group.

## 17. RESCUE OF FALSE MONOSOMIES IN A PGD PROGRAM USING SUBTELOMERIC PROBES

**L. Rodrigo, E. Mateu, A. Mercader, P. Buendía, J. Remohí, A. Pellicer, C. Rubio**

Instituto Valenciano de Infertilidad, Valencia, SPAIN

**OBJECTIVE:** Monosomies appear more frequently than trisomies in preimplantation embryos. The objective of this study is to check if they are true monosomies by using subtelomeric probes directed to different loci of the previously analysed chromosomes.

**DESIGN AND METHODS:** This prospective study includes 301 PGD cycles for aneuploidy screening (January - December 2003). Embryos were biopsied on day 3 and two hybridization rounds were performed for chromosomes 13, 16, 18, 21 and 22 (MultiVysion PB panel, Vysis Inc. Downers Grove, IL, USA) and sex chromosomes (CEP X and CEP Y, Vysis, Inc.). Embryos initially diagnosed as monosomic for chromosomes 13, 16, 18, 21 and 22, were reanalysed using subtelomeric probes for these chromosomes (TelVysion, Vysis Inc.).

**RESULTS:** In total, 235 out of 920 blastomeres were initially diagnosed as monosomic for chromosomes 13 (n=8), 16 (n=142), 18 (n=29), 21 (n=21) or 22 (n=35). After the reanalysis with subtelomeric probes, 122 blastomeres (51.9%) were reconfirmed as true monosomies and 113 blastomeres (48.1%) displayed two hybridization signals.

**CONCLUSIONS:** The use of subtelomeric probes is strongly recommended in a PGD program for aneuploidy screening, in order to improve the accuracy of the diagnosis and to increase the number of euploid embryos available for transfer.

## 18. FETOMATERNAL TRAFFICKING OF CELLS AND NUCLEIC ACIDS: DIAGNOSTIC AND THERAPEUTIC APPLICATIONS

**D.W. Bianchi, P.B. Larrabee, T. Wataganara, K. Khosrotehrani, K.L. Johnson**

Division of Genetics, Departments of Pediatrics and Obstetrics and Gynecology, Tufts University School of Medicine, Boston, MA, USA

To date, the major limitation in prenatal diagnosis using intact fetal cells from maternal blood is the small number of fetal cells in most maternal samples. The study of fetal cells in maternal blood has led to two “spin-off” areas of investigation—the analysis of cell-free fetal DNA and RNA in maternal blood and body fluids, and the study of fetal cells that persist for decades in the mother postpartum (fetal cell microchimerism).

We will present our new data from amniotic fluid (AF) samples that demonstrate that cell-free DNA can be extracted, amplified, and hybridized to DNA microarrays, permitting molecular enhancement of the traditional metaphase karyotype. Analysis by DNA microarray permits detection of any unbalanced chromosome abnormality, including aneuploidy, microdeletions, and sub-telomeric rearrangements. Similarly, we have extracted and amplified cell-free fetal RNA from AF and hybridized the cDNA to Affymetrix microarrays. Results show that global gene expression can be monitored in the living human fetus via amniocentesis. All cell-free fetal RNA in AF derives from the fetus and not placenta.

In another area of investigation, we are exploring the hypothesis that fetal cells that persist in the mother post-partum are novel stem cells (“Pregnancy-associated progenitor cells” [PAPCs]). We breed wild-type female mice to males that are transgenic for the enhanced green Fluorescent protein (GFP+). Half of the pups inherit the transgene and GFP+ fetal cells can be detected by real-time PCR and immunofluorescence. Our preliminary data shows that all pregnant mice have fetal cells in their tissues. Postpartum fetal cells respond to specific types of injury in the mother, suggesting that they behave like stem cells. The field of fetomaternal trafficking continues to expand and provide new insights into fetomaternal biology.

## 19. CELL-FREE FETAL DNA IN MATERNAL BLOOD: MOLECULAR STRUCTURE AND ENRICHMENT APPROACHES

**F.Z. Bischoff**

Department of Obstetrics and Gynecology Baylor College of Medicine, Houston, Texas, USA

Cell-free fetal DNA is present in maternal circulation and amenable to robust recovery. However, the biological (structural) form in which this fetal DNA exists and the mechanisms underlying its variation in plasma are unclear. Thus, to maximize the diagnostic utility of this fetal genetic material, further molecular characterization for improved isolation and/or enrichment of fetal DNA is warranted.

**HYPOTHESIS:** Circulating fetal DNA is unexpectedly stable following venipuncture and cleared rapidly after birth. Thus, the majority of fetal DNA likely circulates in membrane bound vesicles (apoptotic bodies).

**DESIGN AND METHODS:** Determine the biological nature and fundamental properties of circulating fetal DNA. Maternal plasma (n=30; 16 male, 14 female) was separated by centrifugation (x800g) from 12-16 week gestations. Acridine orange (AO), a nucleic acid stain, was used to label recovered plasma, followed by flow cytometric separation of the AO positive non-cellular fraction. Prior to separation, plasma was also subjected to either high speed centrifugation, DNase, Proteinase K or SDS treatment. Specimens were analyzed by fluorescent and electron microscopy as well as real-time PCR to quantify FCY (fetal) and GAPDH sequences.

**RESULTS:** Microscopic analysis revealed presence of apoptotic bodies and nucleosomes in plasma following separation. Flow cytometric evaluation confirmed that DNA is packaged in plasma and high speed centrifugation as well as treatment with DNase and detergents will physically alter this DNA. Real-time PCR quantification demonstrated significant enrichment with mean detection of 15,4 % fetal sequences in sorted compared to 5,3 % in nonsorted specimens.

**CONCLUSION:** We demonstrate that fetal DNA is likely packaged in apoptotic bodies. Based on the observed properties of DNA in plasma, fetal sequence detection can be enhanced 3-5 fold following AO-staining and flow separation.

## **20. FETAL CELLS IN MATERNAL CIRCULATION FOR PRENATAL DIAGNOSIS**

***Y.W. Kan, D. Feng, M-C. Cheung***

Department of Laboratory Medicine, University of California, San Francisco, California, USA

**OBJECTIVE:** To devise a reliable and robust test for recovering fetal nucleated red cells from maternal blood for prenatal diagnosis of single gene disorders such as sickle cell anemia and thalassemia. With such an approach, it will be possible to determine the exact genotype of the fetus.

**DESIGN AND METHODS:** During the past three decades, prenatal diagnosis using DNA analysis has evolved rapidly and has become a routine procedure. In this presentation I will use the hemoglobin disorders to illustrate this evolution in single gene defects and discuss some new technologies that are being developed. Prenatal diagnosis of the hemoglobinopathies initially depends on fetal blood analysis. With the delineation of the genetic defects in these disorders, DNA analysis was first applied with linkage followed by direct analysis as the mutations that cause  $\beta$ -thalassemia were defined. With the introduction of the polymerase chain reaction, diagnosis has become routine. Improvements in several areas are currently under active investigation. Methods of isolating fetal cells from maternal blood are being developed to determine the exact genotype of the fetus. Nucleated fetal red cells are enriched by gradient separation and antibodies directed at them.

**RESULTS:** The cells are then displayed on slides and pure fetal cells recovered for DNA analysis. At present, this method is labor-intensive and not yet suitable for routine analysis. To improve this technology, antibodies specifically directed to fetal red cells are being developed using phage display. Also, automated methods for cell retrieval are being devised. Progress in these areas will be presented.

**CONCLUSIONS:** Although it is feasible to recover fetal cells from the maternal blood, the technique has to be improved to make it a clinically useful procedure.

## **21. RECENT DEVELOPMENTS IN THE BIOLOGY AND DIAGNOSTIC APPLICATIONS OF FETAL NUCLEIC ACIDS IN MATERNAL PLASMA**

***Y.M.D. Lo***

Department of Chemical Pathology, The Chinese University of Hong Kong, Hong Kong, CHINA

The discovery of circulating cell-free fetal DNA and RNA in maternal plasma has opened up numerous avenues of research and potential clinical applications. We have recently demonstrated the utility of such an approach for the non-invasive prenatal exclusion of beta-thalassaemia. Recently we have explored mass spectrometric methods for the detection of single nucleotide differences between circulating fetal DNA and the background maternal DNA. Our group has also developed quantitative methodologies for the measurement of circulating fetal DNA concentrations in health and disease. Such quantitative analysis has demonstrated the elevation of circulating fetal DNA concentrations in conditions such as preeclampsia. With regard to circulating RNA, our group has developed a robust methodology for plasma RNA extraction and detection. We have shown that plasma RNA is surprisingly stable, possibly through the association with particulate matter. We have also shown that a significant proportion of circulating fetal RNA in maternal plasma is of placental origin. Through the use of microarray technologies, the systematic identification of placental mRNA species suitable for non-invasive prenatal diagnosis and monitoring has become possible. We envision that plasma nucleic acids will play an increasingly important role in prenatal research and investigation.

## 22. SAFE- AN EC NETWORK OF EXCELLENCE ON NON-INVASIVE PRENATAL DIAGNOSIS

### **M. Hultén**

Department of Biological Sciences, University of Warwick, UK

Two years ago the European Commission announced its decision to change its research support strategy. Framework 6 recommended relatively large consortia to be formed for either Integrated Projects (IPs) or Networks of Excellence (NoEs) involving around 50-300 scientists. At that time we submitted an Expression of Interest for an IP dealing with Non-invasive Prenatal Diagnosis (NIPD). Following several rounds of negotiations the Commission has now agreed to support the project SAFE with 12 million Euros for 5 years, however as a NoE rather than an IP.

The acronym SAFE stands for Special Non-invasive Advances in Foetal and Neonatal Evaluation Network. This NoE (<http://www2.warwick.ac.uk/cbechat/safe/>) involves 55 partners from 52 institutions (Universities, Hospitals and Small and Medium Enterprises (SMEs)) in 17 countries. Maj Hultén from Warwick University is the Co-ordinator and Jean-Luc Sanne the EC Project Officer. The SAFE NoE is managed by a Steering Committee including the Co-ordinator and the seven Work Package Leaders together with an Advisory Board of 12 internationally outstanding scientists/representatives in the field. The work to be performed by the SAFE NoE will cover wide aspects of non-invasive prenatal diagnosis and neonatal screening. WPs 1 and 2 led by Sinuhe Hahn from Basel and William Clocksin from Oxford involves identification of foetal cells in maternal blood samples, using a variety of cellular markers, together with advanced technology for automatic retrieval. WPs 3 and 4 led by Neil Avent from Bristol and Ciara O'Sullivan from Tarragona concerns in the first instance the introduction and standardisation of DNA technology for non-invasive prenatal diagnosis with respect to conditions such as RH immunisation, beta-thalassaemia and cystic fibrosis as well as the introduction of novel markers with the potential for enhancing all aspects of non-invasive prenatal diagnosis and neonatal screening. WPs 5, 6 and 7 led by Lucia Savadori from Trento, Ala Szczepura from Warwick and Theresa Marteau from London concerns psycho-sociological aspects with particular emphasis on risk estimates, socio-economic as well as ethical issues of crucial importance for the introduction and harmonisation of non-invasive prenatal diagnosis and neonatal screening in Europe.

## 23. DETECTION OF CHROMOSOME 21-ENCODED mRNA OF PLACENTAL ORIGIN IN MATERNAL PLASMA

### **C.B.M. Oudejans<sup>1</sup>, A.T.J.J. Go<sup>2</sup>, A. Visser<sup>1</sup>, M.A.M. Mulders<sup>1</sup>, M.A. Blankenstein<sup>1</sup>, J.M.G. van Vugt<sup>2</sup>**

Departments of <sup>1</sup>Clinical Chemistry and <sup>2</sup>Obstetrics and Gynaecology, VU University Medical Center, Amsterdam, THE NETHERLANDS

**OBJECTIVE:** The presence and detectability of placental RNA in maternal plasma permits rapid screening of new markers to test their feasibility for use in non-invasive prenatal diagnostic assays. This includes markers not accessible by conventional antibody-based assays and markers directly representative for clinical syndromes such as chromosome 21-encoded mRNA for Down syndrome. We challenged these features by testing a large set of placentally expressed genes for their presence in maternal plasma and absence in non-pregnant plasma. **DESIGN AND METHODS:** Total RNA was purified from plasma samples (1.6 ml) of pregnant women (weeks 9-13 of pregnancy) by silica-based affinity isolation and analyzed by RT-PCR with gene-specific primers. Target genes (n=80) were selected for known or expected expression in early placental tissue. This set included genes coding for transcription factors, genes subject to genomic imprinting, non-coding RNA and other genes with restricted or abundant expression in trophoblast cells. Target genes were distributed over all chromosomes except for the Y chromosome. Eight genes tested were located on chromosome 21. **RESULTS:** Out of 80 genes tested, eight genes (10%) were identified with mRNA present and detectable in maternal plasma but absent in non-pregnant plasma. Reliable and reproducible detection in all pregnant females was observed for chromosome 21-encoded mRNA (LOC90625), for 2 genes coding for placenta-specific transcription factors and for 5 genes coding for pregnancy-specific proteins. **CONCLUSIONS:** Expression profiling of placental mRNA in maternal plasma permits rapid screening of novel fetal markers not accessible by antibody-based assays (placental transcription factors) as well as markers directly reflective for clinical syndromes (chromosome 21-encoded mRNA for Down syndrome). Quantification of the latter target gene with calibrator-normalized correction using the former gene might permit the development of diagnostic assays for Down syndrome.

## 24. THE PRESENCE OF MATERNAL DNA IN PERIPHERIAL BLOOD OF NEWBORN INFANTS

**L. Lázár, Z. Bán, Á. Harmath, Z. Papp**

I. Department of Obstetrics and Gynecology, Semmelweis University, Budapest, HUNGARY

**BACKGROUND:** Over the past decade a lot of attention has been directed towards the fetomaternal and maternofetal transfer of nucleated cells and plasma DNA. The effect of fetal cells and plasma DNA in the maternal circulation is unknown, but in some autoimmune skin diseases the fetal DNA is suspected to play an important role in the etiology of the disease. In the same way the presence of maternal cells and plasma DNA in fetal/newborn circulation rise interesting questions. The aim of our study was to detect maternal deoxyribonucleic acid in peripheral blood of premature and mature newborn infants.

**METHODS:** In case of eight RhD-positive mother-RhD negative newborn pairs, peripheral blood samples were collected from the newborn infants within 35-120 minutes after birth. The RhD positive allele was determined by real-time PCR amplification of the exon 7 of the RhD positive allele.

**RESULT:** In all eight cases, maternal DNA was detectable in the peripheral blood of the newborn infants. The result of our study demonstrates that maternal DNA is present in the fetal peripheral blood circulation.

**CONCLUSION:** The presence of maternally derived cells/DNA in blood of newborn infants might have a role in the immunization of the newborn infants.

## 25. CELL-FREE FETAL RNA IN MATERNAL CIRCULATION AS A NEW TOOL FOR THE NON-INVASIVE DETECTION OF ANEUPLOID PREGNANCIES

**S.S.C. Chim<sup>1</sup>, A. El-Sheikhah<sup>2</sup>, N.B.Y Tsui<sup>1</sup>, E.K.O. Ng<sup>1</sup>, R.W.K. Chiu<sup>1</sup>, K.C.A. Chan<sup>1</sup>, Y. Tong<sup>1</sup>, M. Hogg<sup>2</sup>, R. Bindra<sup>2</sup>, T.N. Leung<sup>3</sup>, T.K. Lau<sup>3</sup>, K.H. Nicolaides<sup>2</sup>, Y.M.D. Lo<sup>1</sup>**

Departments of <sup>1</sup>Chemical Pathology and <sup>3</sup>Obstetrics and Gynaecology, The Chinese University of Hong Kong, Prince of Wales Hospital, Hong Kong, CHINA

<sup>2</sup>Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, London, UK

**OBJECTIVE:** To examine any correlation between the relative transcript abundance of placenta-expressed genes and that of cell-free fetal RNA in maternal circulation, and to investigate the feasibility of non-invasive detection of aneuploid pregnancies.

**BACKGROUND:** The recent demonstration of the presence of placental-specific fetal RNA in maternal plasma has opened up new opportunities for non-invasive prenatal investigation. We investigated the correlation of the relative abundance of fetal RNA in maternal circulation with that in the placenta, and explored if one of these RNA transcripts, that coding for the *human chorionic gonadotropin β-subunit (βhCG)*, might be quantitatively different in aneuploid pregnancies.

**DESIGN AND METHODS:** Placental tissue and maternal peripheral whole blood were collected from five pregnancies each of the first and third trimesters. Gene expression profiles were analysed by high-density oligonucleotide microarrays to generate panels of potentially detectable fetal RNA in maternal circulation.

(i) To test for correlation, we selected six transcripts for quantitative RT-PCR analysis in the paired placental tissues and maternal plasma. (ii) To detect for aneuploid pregnancies, we measured the *βhCG* mRNA levels in the maternal serum of 149 pregnant women who presented for aneuploid screening.

**RESULTS:** (i) The relative abundance of cell-free RNA in maternal plasma correlated with that in the placental tissues. (ii) Fetal karyotyping confirmed 15 pregnancies with trisomy 21 and 11 with trisomy 18. The median serum *βhCG* mRNA concentrations of the euploid control, trisomy 21 and trisomy 18 cohorts were 6108, 13165 and 652 copies/ml respectively. Significant differences were observed between the trisomy 18 and control cases (Dunn's test,  $P < 0.05$ ), but not the trisomy 21 and control cases (Dunn's test,  $P > 0.05$ ). Concordant observations were previously found in the placenta from trisomic pregnancies.

**CONCLUSION:** Maternal plasma fetal RNA analysis may represent a new approach for non-invasive prenatal diagnosis.

## 26. NON INVASIVE PRENATAL DIAGNOSIS BY LASER MICRODISSECTION OF HLA-G POSITIVE TROPHOBLASTIC CELLS IN TRANSCERVICAL SAMPLES AND QF-PCR

**V. Cirigliano<sup>1</sup>, J. Bulmer<sup>2</sup>, R. Cioni<sup>3</sup>, F. Sole<sup>4</sup>, C. Costa<sup>4</sup>, M. Adinolfi<sup>5</sup>**

<sup>1</sup>General Lab, Barcelona, SPAIN, <sup>2</sup>Royal Victoria Infirmary, Newcastle-upon-Tyne, UK, <sup>3</sup>University of Firenze, ITALY, <sup>4</sup>Hospital del Mar, Barcelona, SPAIN, <sup>5</sup>University College London, UK

**OBJECTIVE:** To assess the frequency of cytotrophoblastic cells in endocervical samples collected at early stages of gestation using a specific anti-HLA-G McAb (G233) and their suitability for non invasive prenatal diagnosis. In selected samples, cells identified by immunostaining were isolated by laser microdissection and tested by quantitative fluorescent PCR (QF-PCR) for the presence of paternal DNA markers, in order to establish their fetal origin.

**DESIGN AND METHODS:** Syncytial and cytotrophoblastic cells from 23 transcervical samples were identified by immunostaining with McAb G233 reacting against HLA-G antigen and with antibodies against cytokeratin. Slides from the same samples were also tested by fluorescent *in situ* hybridization (FISH), while selected samples were analysed by QF-PCR. Four samples retrieved from mothers with male fetuses were immunolabelled and then cytotrophoblastic cells, syncytial fragments and maternal epithelial cells were collected by PALM laser microdissection and tested by QF-PCR.

**RESULTS:** All samples retrieved from mothers with male fetuses were found to contain cells with chromosome Y-specific signals when tested by FISH. Using McAb anti-HLA-G, cytotrophoblastic cellular elements were detected in about 50% of the samples. From four samples, cellular elements identified by immunostaining as cytotrophoblast or syncytial fragments were collected by laser microdissection and shown to be of fetal origin when tested by QF-PCR for the presence of fetal DNA markers.

**CONCLUSIONS:** These results confirm that, at early stage of gestation, fetal cells are released in the lower uterine cavity and that they can be isolated and analysed for non or minimally invasive prenatal diagnosis of single gene defects and common chromosome aneuploidies.

## 27. DNA IDENTIFICATION OF FETAL CELLS ISOLATED FROM CERVICAL MUCUS: POTENTIAL FOR EARLY NON-INVASIVE PRENATAL DIAGNOSIS

**M. Katz-Jaffe<sup>1,2</sup>, D. Mantzaris<sup>2</sup>, D. Cram<sup>1,2</sup>**

<sup>1</sup>Monash IVF, <sup>2</sup>Monash Institute of Reproduction and Development, Melbourne, Victoria, AUSTRALIA

**OBJECTIVE:** Continuing research in the field of prenatal diagnosis aims towards early, rapid and less invasive techniques that could be performed during the first trimester of pregnancy to detect chromosomal abnormalities and single gene disorders. The aim was to develop a reliable method to isolate sufficient fetal cells for genetic diagnosis.

**DESIGN AND METHODS:** A total of 60 pregnant women were recruited for the study prior to an elective termination of pregnancy (7-12 weeks gestation). Cervical mucus was aspirated from just above the internal os. By multiplex fluorescent PCR, fetal identity was confirmed by comparing allelic profiles with those from maternal cells.

**RESULTS:** A non-invasive method to isolate fetal cells from aspirated cervical mucus of pregnant women was developed using a combination of mechanical and enzymatic digestion, fluorescent immunohistochemistry, micromanipulation and single cell DNA allelic profiling. Fetal cells were consistently retrieved as early as 7 weeks gestation.

**CONCLUSIONS:** This novel non-invasive method is rapid and efficient with results attainable within 24 hours. The technique would offer earlier reassurance and the option of first trimester therapeutic abortions to both high and low risk pregnant women.

## 28. ANALYSIS OF FETAL ERYTHROCYTES IN MATERNAL BLOOD FOR PRENATAL DETECTION OF HB BART'S DISEASE

*E.T. Lau<sup>1</sup>, Y.K. Kwok<sup>2</sup>, D.H.K. Chui<sup>3</sup>, H.Y. Luo<sup>3</sup>, K.Y. Leung<sup>1</sup>, C.P. Lee<sup>1</sup>, Y.H. Lam<sup>2</sup>, M.H.Y. Tang<sup>1</sup>*

<sup>1</sup>Prenatal Diagnostic and Counselling Department, Tsan Yuk Hospital, <sup>2</sup>Department of Obstetrics and Gynecology, University of Hong Kong, Hong Kong, CHINA

<sup>3</sup>Departments of Medicine and Pathology, Boston University School of Medicine, Boston, MA, USA

**OBJECTIVE:** We investigate a non-invasive technique for early prenatal detection of Hb Bart's Disease using dual color immunofluorescence antibody staining of erythrocytes from maternal blood.

**DESIGN AND METHODS:** Alpha thalassemia is the most common monogenic disease in Southeast Asia with 4.5% of the population in Hong Kong being heterozygous carriers of the Southeast Asian type deletion (-SEA). Hb Bart's disease, the severe form of  $\beta$ -thalassemia, is characterized by the loss of all 4  $\beta$ -globin genes resulting in the absence of  $\beta$ -globin chain production. As fetal cells are present in maternal blood, we would like to identify fetal Bart's cells in maternal circulation. Fetal cells affected by Hb Bart's disease can be marked by the absence of  $\beta$ -globin expression among  $\beta$ -globin producing maternal cells. In this study, maternal blood smears from 8 known Hb Bart's pregnancies and 30 at-risk pregnancies (between 7 to 15 weeks of gestation) were used for dual color immunofluorescence staining against  $\beta$ - and  $\beta$ -globin antibodies.

**RESULTS:** Fetal Bart's erythrocytes stained with anti- $\beta$  but not with anti- $\beta$  globin antibodies were identified in all affected pregnancies (18 cases). One to 5  $\beta$ -negative erythrocytes were detected per smear, and the earliest affected sample was at 10 weeks gestation. In one of the 20 unaffected pregnancy, one  $\beta$ -negative erythrocyte was found at 9 weeks, but not at 14 weeks gestation.

**CONCLUSIONS:** The sensitivity of the immunofluorescence antibody staining technique is 100%, with at least one fetal non-nucleated red cell found in 3111 of maternal blood. Results showed that this non-invasive method could detect Hb Bart's disease during early pregnancy prior to possible detection by ultrasonography. Efforts are directed to enrich the  $\beta$ -negative blood cells.

## 29. SEX DETERMINATION BY THE DETECTION OF SRY REGION WITH REAL-TIME PCR IN MATERNAL PLASMA

*L. Lázár, Z. Bán, B. Nagy, A. Beke, J. Rigó, Z. Papp*

I. Department of Obstetrics and Gynecology, Semmelweis University, Budapest, HUNGARY

**OBJECTIVE:** Non-invasive methods using maternal plasma for molecular genetic diagnosis became an important field of interest in prenatal diagnosis. Detection of free fetal DNA in maternal plasma with real-time PCR has been shown to be useful for fetal gender determination. The aim of our study was to determine the fetal sex with the real-time PCR amplification of the SRY region from maternal plasma, to control the sensibility and specificity of the method.

**METHODS:** Maternal plasma before amniocentesis, and amniotic fluid samples were obtained from 70 pregnant women. Real-time PCR analysis of the SRY region was performed in order to determine the fetal sex. Routine karyotyping was also performed on the samples.

**RESULTS:** We found male fetuses in 36 of 70 pregnancies by cytogenetic analysis. Real time PCR of maternal plasma has been positive for the SRY region in 38 cases. In 66 cases the cytogenetic gender and the real-time PCR results were correlating. In one case of 46,XY karyotype the PCR reaction for SRY region was negative, in tree cases of SRY positivity the karyotype was 46,XX.

**CONCLUSIONS:** The result of our study suggest that real time PCR detection of SRY region is a possibile method for non-invasive fetal gender determination, and could be an important prenatal diagnostical method in case of diseases showing X-linked inheritance.

### **30. METHOD FOR STANDARDIZATION OF FETAL DNA DETECTION AND QUANTIFICATION FROM MATERNAL BLOOD / PRELIMINARY STAGE OF AN INTERNATIONAL MULTICENTRIC STUDY**

**A. Levy-Mozziconacci<sup>1,6</sup>, B. Poneillé<sup>1</sup>, F. Muller<sup>2</sup>, F. Roux<sup>3</sup>, H. Chaudet<sup>4</sup>, J. Mancini<sup>5</sup>, C. D'Ercole<sup>6</sup>, L. Boublil<sup>6</sup>, J. Gabert<sup>1</sup>**

Laboratoire de Biochimie et Biologie Moléculaire<sup>1</sup>, DIM Nord<sup>5</sup>, Service de Gynécologie-Obstétrique<sup>6</sup>, CHU Nord, Service de Médecine nucléaire<sup>3</sup> CHU Timone DIM<sup>6</sup> Hopitaux Sud, Marseille, FRANCE, Laboratoire de Biochimie et Hormonologie<sup>2</sup>, CHU R. Debré, Paris, FRANCE

**OBJECTIVE:** Analysis of fetal DNA from maternal blood by real-time quantitative PCR ( RQ-PCR) offers great potential for non invasive prenatal genetic diagnosis. The detection of male specific DNA sequences or the unique gene sequence such as the RhD locus has been used. The quantity of fetal DNA in maternal blood has been also used as a marker of genetic disorders and complications of pregnancy. Actually, different data are typically generated by a single laboratory under specific conditions and often include data from a small number of patients. To prove the impact of this technology, multicentric study are indispensable. We propose a methodology for standardization of the quantification of fetal DNA in maternal blood.

**DESIGN AND METHODS:** For that, we have based on an original approach of calibration already developed in our laboratory (Gabert et al., Leukemia, 2003; 17, 2318) wich consists in manufacturing plasmid DNA calibrators containing the target gene sequence. Each maternal serum or plasma sample is compared with a range of dilution obtained from the construction of these plasmids. Two plasmids are used: one containing a sequence of beta-globine gene to quantify total DNA and the other containing a sequence of the gene SRY (Y chromosome) to quantify fetal male DNA. We reported the analysis of 75 patients ( serum and plasma).

**RESULTS:** Fetal DNA and total DNA are also quantified in 19 serum and 12 plasma from women with 21 trisomy foetus (gestational age from 15 to 17 weeks). Cell-free fetal DNA levels are increased only in maternal plasma.

**CONCLUSIONS:** We have developed robust calibrators to standardize the step of RQ-PCR analysis and to perform intra and interlaboratory comparison of fetal DNA detection or quantification from maternal samples. The stability of these calibrators will permit to develop standardization and quality control programs in a large multicenter study. This approach is indispensable to develop this new technology in clinical laboratories.

### **31. FIRST TRIMESTER NRBC COUNT IN MATERNAL CIRCULATION: CORRELATION WITH DOPPLER ULTRASOUND STUDIES**

**A. Mavrou<sup>1</sup>, A. Kolialexi<sup>1</sup>, A. Souka<sup>2</sup>, A. Pilalis<sup>2</sup>, Y. Kavalakis<sup>2</sup>, P. Antsaklis<sup>2</sup>, E. Kanavakis<sup>1</sup>, A. Antsaklis**

<sup>1</sup>Medical Genetics and <sup>2</sup>I. Department of Obstetrics and Gynecology, Athens University School of Medicine, Athens, GREECE

**OBJECTIVE:** Impaired placental perfusion is associated with increased feto-maternal cell trafficking, preceding PET/IUGR. The study aimed to determine whether the No. of Nucleated Red Blood Cells (NRBCs) in maternal circulation, in the 1<sup>st</sup> trimester of pregnancy, could predict pregnancies that will have an anomalous Doppler in the 2<sup>nd</sup> trimester.

**DESIGN AND METHODS:** 85 blood samples were obtained from women at 11-14 weeks of gestation with mean uterine arteries PI>1.6 as noted by Doppler ultrasonography. NRBCs were enriched by MACS using anti-CD71 conjugated microbeads and enumerated after May-Grunwald / Giemsa staining.

**RESULTS:** An average of 4.8 NRBCs (range 1-75) were identified in 68 cases. Follow up scans at 22-24 weeks were available in 46 cases. In 39 of those blood flow in the uterine arteries normalized, whereas in 7 women high resistance uterine blood flow was noted (mean PI>1.6). One woman of the high resistance group developed PET (4 NRBCs) and another delivered a growth-restricted baby (75 NRBCs). The average No. of NRBCs in the 1<sup>st</sup> trimester was similar in the women that subsequently normalized their Doppler indices and in those that continued to have increased impedance (X vs Y respectively).

**CONCLUSIONS:** The study indicates that the No of NRBCs in maternal corculation in the 1<sup>st</sup> trimester cannot be used as a second line screening to identify pregnancies at high risk for PET/IUGR. High impedance blood flow in the uterine arteries in the 1<sup>st</sup> trimester may be due to unfinished process of trophoblastic invasion, most likely to be completed successfully by 22-24 weeks.

### **32. OUR FIRST STEPS IN DETECTING FETAL CELLS IN THE MATERNAL CIRCULATION FOR PRENATAL DIAGNOSIS**

**Gy.R. Nagy<sup>1</sup>, Z. Bán<sup>1</sup>, F. Sipos<sup>2</sup>, J. Oroszné Nagy<sup>1</sup>, A. Beke<sup>1</sup>, Z. Papp<sup>1</sup>**

I. Department of Obstetrics and Gynecology, <sup>2</sup>II. Department of Internal Medicine, Semmelweis University, Budapest, HUNGARY

**INTRODUCTION:** The isolation and analysis of fetal cells in maternal blood during pregnancy is under investigation as a means of noninvasive prenatal diagnosis. The aim of our study was to detect fetal gender from maternal peripheral blood samples during pregnancy with the detection and analysis of fetal nucleated red blood cells. Here we report our first results.

**DESIGN AND METHODS:** We obtained maternal blood from 14 singleton pregnancies. After a double density Percoll gradient separation, magnetic activated cell sorting was performed by positive selection for nucleated red blood cells with anti-CD71. With the help of this enrichment step, followed by immunophenotyping with an anti-haemoglobin-epsilon monoclonal antibody, the isolation of the epsilon haemoglobin chain positive cells with micromanipulation could be done. We performed single cell fluorescent PCR analysis of these cells; we used primers for the amelogenin gene to detect fetal gender. In 12 cases we could compare our findings with the results of amniocentesis.

**RESULTS:** Fetal gender was successfully determined in 9 out of 12 cases, among them in 2 cases with Klinefelter syndrome.

**CONCLUSION:** The results of our study suggest that the method described above can be useful in prenatal diagnosis to detect fetal gender, and should be improved to detect chromosomal abnormalities.

### **33. DEVELOPMENT OF MONOCLONAL ANTIBODIES FOR THE DIFFERENTIATION OF FETAL AND ADULT ERYTHROID BLOOD CELLS**

**S. Zimmermann, C. Hollmann, S. Stachelhaus**

Department of Prenatal Diagnosis, AdnaGen AG, Langenhagen, GERMANY

**OBJECTIVE:** The lack of markers, which specifically identify fetal cells is the crucial obstacle for the development of a reliable non-invasive prenatal diagnostic in maternal blood. The aim of this approach was to generate monoclonal antibodies that label exclusively fetal erythroid cells and thus allow a differentiation of fetal and adult blood cells.

**DESIGN AND METHODS:** Mice were immunized with flow sorted human cord blood cells (CD71+, antigen-i+, CD19- and CD45-). Hybridoma supernatants were screened on pooled mononuclear cord blood cells, whereas the corresponding amount of erythroid precursors was determined by cytochemical staining of blood smears. For the hybridoma screening a six-parameter flow cytometric analysis (four colours, forward and side scatter) was set up for the simultaneous identification of erythroid precursor cells, leukocytes, enucleated erythrocytes and for antibodies reacting specifically with fetal cells. Furthermore, immunohistochemical analyses have been performed with fetal blood smears and fetal liver sections from the 6<sup>th</sup> up to 38<sup>th</sup> week of gestation as well as with adult blood, normal adult bone marrow and adult erythrocytes as controls.

**RESULTS:** A clone with specificity for a surface antigen exclusively expressed on fetal erythroid cells has been identified. The new antibody showed unaltered binding to erythroid cells from fetal blood of early times of gestation (6<sup>th</sup> week) up to childbirth. Moreover, detailed examinations showed no surface reactivity with adult erythrocytes, erythroblasts or lymphatic and myeloid cells. This antibody did not react with cells of fetal haemolymphatic organs.

**CONCLUSIONS:** The investigations showed that the new monoclonal antibody binds specifically fetal erythroid cells and thus can differentiate between fetal and adult red blood cells. Because of the expression of this fetal antigen in early stages of gestation a non-invasive prenatal diagnostic may be feasible. This antibody can be applied for different enrichment techniques and/or for the identification of fetal erythroid cells.

### **34. PRENATAL DIAGNOSIS OF SINGLE GENE DISORDERS**

#### ***F. Fiorentino***

"GENOMA"- Molecular Genetics Laboratory. "Embryogen" - Preimplantation Genetic Diagnosis Centre, Rome, ITALY

Traditionally, the prevention of genetic diseases in patients who are at substantial risk of conceiving children affected by a known genetic defect has been achieved by prenatal diagnosis, using mainly chorionic villus sampling (CVS), amniocentesis and cordocentesis. This approach aims to establish the presence or absence of the disease to avoid the birth of affected children.

The technologies developed for the Human Genome Project, the recent surge of available DNA sequences resulting from it and the increasing pace of gene discoveries and characterization have all contributed to new technical platforms that have enhanced the spectrum of disorders that can be diagnosed prenatally. DNA-based testing is becoming possible for an increasing number of hereditary diseases as the responsible genes are mapped to individual chromosomes and then isolated and characterized.

The introduction of automated fluorescence-based DNA analysis methods provided a fast and accurate way for prenatal diagnosis of single gene disorders. The use of fluorescent PCR, direct sequencing and minisequencing techniques, has increased accuracy and reliability of the analysis, due to the higher sensitivity of the approach. Furthermore, automation and computerized analysis systems has increased the overall analysis throughput.

Preimplantation genetic diagnosis (PGD) represents a very early form of prenatal diagnosis aimed at detecting genetic diseases on IVF embryos before their transfer into the uterus. PGD has revealed an acceptable alternative to prenatal diagnosis, mainly in those countries where pregnancy interruption is forbidden by law. or for couples where termination of pregnancy represents a religious and/or ethical problem.

Using the above approaches, over 15.000 prenatal genetic tests and 130 PGD cycles have been performed in our department. The overall experience of using prenatal and preimplantation genetic diagnosis of single gene disorders presently exceeds 30 different diseases.

Finally, the completion of the genome project combined with the recent advances in nanotechnologies, are enhancing the development of new diagnostic methods, such as microarrays (DNA chips), for rapid, accurate and simultaneous mutation detection. The next few decades hold the promise of many more advances in preimplantation and prenatal genetic testing.

### **35. PRENATAL DIAGNOSIS OF GENETIC METABOLIC DISORDERS**

#### ***W.J. Kleijer***

Department of Clinical Genetics, Erasmus MC, Rotterdam, THE NETHERLANDS

We have made 2400 prenatal enzyme analyses for 60 different metabolic disorders with 520 affected fetuses (22%). Generally the enzymatic approach is reliable, fast and available to all families at risk as long as the enzyme defect in the index patient has been shown. For some disorders with less reliable enzyme results DNA-mutation analysis is preferred (e.g. Canavan, Sialic acid storage, Menkes and Niemann-Pick type C disease). In addition, mutation analysis may occasionally be wanted if low heterozygote enzyme activity prevents a definite diagnosis (e.g. Krabbe and Hurler disease). However DNA-diagnostic laboratories cover only part of these rare disorders and it appears not always possible to trace the mutations timely.

Our finding of 22% affected fetuses is somewhat below the 25% expected if there had been a 1:4 risk in all pregnancies. After subtracting all cases for X-linked diseases, because this group includes many female relatives who are possible but not obligate carriers, the percentage of affected fetuses rises to 24%. Also for most individual diseases the percentage of positive diagnoses is close to 25%. The exception however is citrullinemia for which we diagnosed 35 affected fetuses in 90 pregnancies at risk, i.e. 39%, which seems an exceedingly unlikely high rate. We have considerable evidence that this is not caused by technical error leading to false-positive diagnosis, while in all cases (n=8) in which cells of the affected fetus were available the same mutations as in the previous affected child in the family was demonstrated. Speculatively one might explain the results by preferential transmission of the mutated ASS allele.

## **36. 20 YEARS EXPERIENCE OF PRENATAL DIAGNOSIS OF HEMOGLOBIN DISORDERS BY DNA ANALYSIS**

### ***J. Old***

National Hemoglobinopathy Reference Laboratory, Churchill Hospital, Oxford, UK

The National Hemoglobinopathy Reference Laboratory has performed more than 2800 prenatal diagnoses of hemoglobin disorders by DNA analysis of chorionic villus DNA since 1982. The diagnoses were for  $\beta$ -thalassaemia (42%), sickle cell disease (56%) and alpha-thalassaemia (2%). Since 1990 all diagnoses except those for  $\beta$ -thalassaemia have been performed by polymerase chain reaction (PCR) techniques. To conform to best practice guidelines, mutations are diagnosed by two different techniques when ever possible.

For the diagnosis of  $\beta$ -thalassaemia, our strategy is to screen first for the common known mutations found in the ethnic group of the patient by the amplification refractory mutation system (ARMS). Rare or new mutations are identified by direct DNA sequencing. Prenatal diagnosis for 38 different  $\beta$ -thalassaemia mutations have has been carried out by this strategy. All diagnoses performed by ARMS-PCR are confirmed by DNA sequence analysis. The diagnosis of sickle cell disease is carried out by ARMS-PCR and restriction enzyme digestion of PCR products (RE-PCR) by Dde1. Hb C, D Punjab and Hb E mutations are diagnosed by ARMS-PCR and confirmed by RE-PCR or DNA sequencing. Alpha-thalassaemia is diagnosed by the technique of multiplex gap-PCR. Data for the last four years recorded 89 affected fetuses from 354 diagnoses (25.1%). A check for maternal contamination of fetal DNA is carried out by amplification of variable number tandem repeat polymorphisms (VNTRs) in the fetal, maternal and paternal DNAs. We use the Apo B, IgJh, Co12A1 and D4S95 VNTR loci, which allow separation of the products by simple agarose gel electrophoresis. However they are not informative in every case, and we have found the vWF Intron 40 VNTR, analysed by amplification with fluorescently-labeled primers and separation on a capillary-based genetic analyser, to be more informative for all ethnic groups at risk for  $\beta$ -thalassaemia. Despite such precautions diagnostic errors have occurred for a number of reasons, including maternal DNA contamination, mispaternity, amplification failure and misreferral of parental phenotypes. The latter is a particular problem with the prenatal diagnosis of sickle cell disease, which often has to be performed without a sample from the father. There have been five diagnostic errors by Southern blot analysis and five by PCR techniques, giving a laboratory diagnostic error rate of 0.56%.

## **37. PRENATAL DIAGNOSIS AND TREATMENT OF CONGENITAL ADRENAL HYPERPLASIA DUE TO 21-HYDROXYLASE DEFICIENCY**

### ***L. Otano***

Division of Obstetrics, Unit of Fetal Diagnosis and Treatment, Hospital Italiano de Buenos Aires, Buenos Aires, ARGENTINA

Congenital adrenal hyperplasia (CAH) caused by 21-hydroxylase (21-HO) deficiency in its classical forms (simple virilizing and salt wasting) is an autosomic recessive disorder caused by mutation of the CYP21 gene with a prevalence of approximately 1 in 16.000 births in most populations. Female affected fetuses are exposed to high levels of adrenal androgens from the seventh week of gestation, resulting in a newborn with ambiguous genitalia. The molecular analysis of the CYP21 gene in the affected child and in the parents has become a key tool for the management of pregnancies at risk for CAH. Most mutations arise from two types of recombination between CYP21 gene and CYP21P (pseudogene) by a process termed "gene conversion" resulting in a number of different deleterious mutations and deletions. Prenatal diagnosis and treatment of CAH with dexamethasone has been successfully used to prevent virilization of the external genitalia in affected females. For an adequate management of a pregnancy at risk for CAH, it is crucial to give a comprehensive genetic counseling to the couple and to perform the molecular studies of the parents and the index case preconceptionally. Odds are that only one in eight fetuses will be an affected female when both parents are carriers. To prevent genital virilization, dexamethasone must be administered early in the first trimester to all women with pregnancies at risk. So, to minimize the prenatal exposure to unnecessary treatment, a prompt and accurate prenatal genetic diagnosis is crucial. If the fetus is a male or an unaffected female, the treatment must be stopped. Our experience in the prenatal diagnosis of CAH during the first trimester in chorionic villi and treatment on pregnancies at risk will be presented. Different issues in the management of such pregnancies will be reviewed and discussed, such as genetic counseling, molecular genetics of CAH, benefits and concerns of dexamethasone prenatal treatment, prenatal diagnosis procedures, pitfalls in molecular studies, novel approaches (for instance, fetal free DNA in maternal plasma), ultrasound markers (increased nuchal translucency, enlarged fetal adrenals).

### **38. THE SOONER WE KNOW...?**

***E. Pergament, M. Fiddler***

Northwestern Reproductive Genetics, Inc., Chicago, Illinois, USA

The readily understood paradigm of genes as determinants of diseases and traits is giving way to probabilistic models that require new insights into how biological and non-biological variables – genes and environment – truly interact over time to influence both normal, well-functioning people and individual differences, including common disorders and diseases. Prenatal diagnosis (PD) has developed over the past 30 years in conjunction with the identification of disease-determining genes and has been successful as an expression of the prevailing paradigm. The recent application of PD to adult onset, single gene conditions has stretched the boundaries of commonly accepted norms. As probabilistic and interactive models of development become articulated and supported by data, the role and purpose of prenatal diagnosis is likely to be reconceived, leading to decisions as to whether PD for traits as well as diseases expressed at any point in postnatally – from infancy through adulthood – should become part of the repertoire of prenatal diagnosis. This presentation will organize into categories examples of diseases and conditions identified as targets for early diagnosis and will then assess the prospects for their inclusion as well as many others in the repertoire of prenatal diagnosis. Among the illustrations will be Huntington's disease, Parkinson's disease (e.g. Brugada syndrome, low birthweight), diabetes, correlates of learning capabilities (e.g. CREB family genes, BDNF), early-onset Alzheimer disease (e.g. presenilin genes) and behavioral disorders (e.g. autism spectrum; ADHD). While the genetic basis for some is well established and relatively "simple", others are considerably more complex as the outcome of multiple epigenetic processes. On the assumption that procedural risks for prenatal diagnosis will be eliminated with new techniques and that the depth of knowledge regarding influences on paths of normal and aberrant development will increase, these will be examined with two basic questions in mind: "Is there an appropriate role for prenatal diagnosis of complex conditions?" and, if so " Under what circumstances?".

### **39. PRENATAL ULTRASOUND SCREENING OF MOST COMMON HEREDITARY DISORDERS IN LOW-RISK PREGNANT WOMEN POPULATION**

***F. Stipoljev, A. Kurjak***

Department of Obstetrics and Gynecology, Medical School University of Zagreb, Hospital "Sveti Duh", Zagreb, CROATIA

**OBJECTIVE:** Molecular genetics has been rapidly developed to provide useful tools for antenatal and postnatal diagnosis of gene abnormalities, detection of carriers and modified carrier risk. The introduction of fetal ultrasonography in the routine practice, increased the number of genetic disorders which can be diagnosed in the embryonic and fetal period. Some of them have the higher incidence rate comparing to incidence of common chromosomal diseases such as trisomy 18 and trisomy 13. The frequency of specific anomalies vary primarily depending on the availability of imaging technology and on the skills of sonographer.

**METHODS AND RESULTS:** Cystic fibrosis is most common autosomal recessive disease with the carrier frequency of around 1 in 25. In our group of 16 second-trimester fetuses with isolated hyperechogenic bowel, direct DNA CFTR analysis from amniotic fluid cells revealed one fetus to be heterozygote carrier, and one affected homozygote for DF508 mutation. Spinal muscular atrophy is the second most common lethal autosomal recessive disorder of the childhood with carrier frequency of 1 in 50. We also presented two prenately diagnosed cases of arthrogryposis multiplex congenital (AMC) evaluated by 3D sonography. The diagnosis was made by the 24 and 25 weeks gestation, respectively. Ventricular septal defect was also detected in one fetus. Genomic DNA was obtained from fetal blood. The deletions in survival motor neuron gene exons 7 and 8, and exon 5 of neuronal apoptosis inhibitory protein gene are not found. Even not confirmed in our two cases, the presence of deletions in survival motor neuron gene were found in other cases of isolated AMC or associated with the congenital heart disease.

**CONCLUSIONS:** Three dimensional sonography provides additional information in the precise evaluation of fetal morphology and enables specific diagnosis of associated fetal malformations. Correct diagnosis is of importance as accurate genetic counseling is fully dependent of it. The finding of specific ultrasonographic markers indicates the necessity for further prenatal molecular testing, which is of great importance in low-risk population.

#### **40. FETAL LOSS, CLUBFOOT DEFORMITY AND EARLY AMNIOCENTESIS:**

##### **A COMPARISON OF EARLY AMNIOCENTESIS AND LATE CHORIONIC VILLUS SAMPLING IN AN INTERNATIONAL RANDOMIZED STUDY**

**J. Philip**

Research Unit for Prenatal Diagnosis, Rigshospitalet, Copenhagen, DENMARK

**OBJECTIVE:** The safety and accuracy of transabdominal chorionic villus sampling (CVS) and amniocentesis at 13 and 14 gestational weeks have not been investigated in randomized trials, yet prenatal diagnosis in this time interval has become increasingly relevant due to early trisomy screening.

**DESIGN AND METHODS:** We compared early amniocentesis with late CVS from 91-104 days gestation in a randomized trial in a predominantly advanced maternal age population. Before randomization the feasibility of both procedures was confirmed by ultrasound and experienced operators performed sampling under ultrasound guidance; conventional cytogenetic analysis was employed. The primary outcome measure was a composite of fetal loss plus preterm delivery before 28 gestational weeks in cytogenetically normal pregnancies.

**RESULTS:** We randomized 3775 women into two groups (1914 to CVS; 1861 to amniocentesis) which were comparable at baseline. More than 99.6% had the assigned procedure and 99.9% were followed through delivery. In the cytogenetically normal cohort (3698), there was no difference in primary study outcome: 2.1% for late CVS and 2.3% for early amniocentesis. However, spontaneous losses before 20 weeks and procedure-related, indicated terminations combined were increased in the early amniocentesis group ( $p=0.07$ , relative risk 1.74). We found a four-fold increase in the rate of talipes equinovarus after early amniocentesis ( $p=0.02$ ) overall, and in week 13 ( $p=0.03$ , relative risk=4.65), but data were insufficient to determine this risk in week 14.

**CONCLUSIONS:** Early amniocentesis at 13 weeks carries a significantly increased risk of talipes equinovarus compared with CVS and also the suggestion of an increase in early, unintended pregnancy loss.

#### **41. MATERNAL AND FETAL LYMPHOCYTE SUBPOPULATIONS IN CMV INFECTION**

**U. Nicolini** (Milano, Italy): Abstract not received

#### **42. PRENATAL DIAGNOSIS OF CONGENITAL INFECTIONS**

**Y. Ville** (Poissy, France) Abstract not received

#### **43. PRENATAL DIAGNOSIS OF HERLITZ JUNCTIONAL EPIDERMOLYSIS BULLOSA**

**M. Csikós<sup>1,2</sup>, E. Rácz<sup>1,2</sup>, R. Benkő<sup>1,2</sup>, A. Bóna<sup>1,2</sup>, Z. Bán<sup>3</sup>, A. Beke<sup>3</sup>, Z. Papp<sup>3</sup>, A. Horváth<sup>1</sup>, S. Kárpáti<sup>1,2</sup>**

<sup>1</sup>Department of Dermato-Venereology and Skin-oncology, Semmelweis University, Budapest, HUNGARY, <sup>2</sup>Hungarian Academy of Sciences-Semmelweis University Dermatological Research Group of Genetics and Immunology, Budapest, HUNGARY, <sup>3</sup>I. Department of Obstetrics and Gynecology, Semmelweis University, Budapest, HUNGARY

**OBJECTIVE:** Herlitz junctional epidermolysis bullosa (H-JEB, OMIM 226700) is a lethal, autosomal recessive disorder characterized by blister formation at the level of the lamina lucida within the cutaneous basement membrane zone. H-JEB is frequently associated with premature-termination-codon (PTC) mutations in both alleles of one of the three genes *LAMA3*, *LAMC*, or *LAMB3* encoding the subunit polypeptides alpha3, beta3, gamma2 of laminin 5. The majority of the laminin 5 mutations reside in *LAMB3* gene on chromosome 1 (GeneBank accession number L25541).

**DESIGN AND METHODS:** The newborn male from healthy, non-consanguineous parents had extensive blistering and demonstrated negative immunofluorescence staining for laminin 5 beta3 chain, and revealed tissue separation within lamina lucida of the dermal-epidermal junction, diagnostic for H-JEB. Mutation analysis was performed by amplification of genomic DNA with PCR using *LAMB3*-specific primers, heteroduplex analysis, direct nucleotide sequencing. Mutation verification restriction endonuclease digestion (BglII) was applied.

**RESULTS AND CONCLUSIONS:** We detected a heterozygous hotspot PTC mutation 1903C>T, R635X. The mother of the proband was found to be a heterozygous carrier for this mutation, whereas the mutation in the father remained unknown.

Nonpaternity was excluded by use of microsatellite markers from different chromosomes. Based on these results, DNA-based prenatal diagnosis was performed by chorionic villus sampling for subsequent second pregnancy in the family. The fetus was found to be negative of the R635X mutation, indicating that they was phenotypically unaffected. Haplotype analysis with intragenic *LAMB3* polymorphisms and using microsatellite markers surrounding the *LAMB3* gene showed that the first patient and the fetus carry the same maternal and paternal chromosome 1/*LAMB3* haplotype, without R635X in the fetus. In that case, an unaffected child was predicted and the mother gave birth to a healthy male. We concluded that the mutation is most likely present in a percentage of the maternal germline, responsible for this unusual mode of inheritance in H-JEB.

#### **44. PRENATAL DIAGNOSIS AMONG HIV INFECTED PREGNANT WOMEN**

**S.S. Hernandez, A. Suy, S. Pisa, A. Borrell, O. Coll**

Institut Clínic de Ginecologia, Obstetrícia i Neonatologia, Hospital Clínic, Universitat de Barcelona, SPAIN

**OBJECTIVE:** HIV infection is considered a contraindication for invasive procedures to determine fetal karyotype. Nevertheless some of these women are at high risk for chromosomal abnormalities.

**DESIGN AND METHODS:** HIV infected women who delivered or terminated their pregnancy between 6/2002 and 12/2003 in our institution. All women were offered first (NT + PAPP-A and free  $\beta$ -HCG) or 2<sup>nd</sup> trimester screening ( $\beta$ -HCG + AFP). An amniocentesis was offered if: risk for Down Sdr.  $>1/250$ , maternal age  $\geq 38$  years, presence of an ultrasound marker or if family inherited disorder. HIV viral load and CD4 cell counts were determined at first prenatal visit and before amniocentesis was offered. Risk benefits of the procedure were discussed with patients.

**RESULTS:** A total of 117 pregnancies (1 set of twins) among 112 women were evaluated. Maternal characteristics are as follows: median age 35 years (range 18-42), nulliparous: 50,4%, caucasian 82,9%, sexually acquired HIV: 18%, months from diagnosis: 2 to 232. A coinfection with HCV and HBV was present in 40,7% and 4,3% respectively. In 88% of pregnancies first prenatal visit occurred  $\leq 18$  weeks. First and/or second trimester screening was performed in 82,9% of cases. In 13 cases (11,1%) an invasive procedure was recommended (abnormal result: 5, US marker: 4, maternal age 3, genetic anomaly 1). After specific counseling, 3 patients declined amniocentesis. Of the other 10: viral load was  $<300$  copies/ml in 8, and 2 were below 10.000. In all but one, CD4 were  $>400$  cells/ml. All patients were under  $\geq 3$  antiretrovirals. Placental passage could be avoided in all but one case. Two abnormal karyotypes were obtained (47XY +21 and 45 XO) and pregnancies were terminated. One of the fetuses with a normal karyotype and malformations died at 17 weeks. None of the 7 infants with normal karyotypes fully evaluated are HIV infected after 3 months of delivery.

**CONCLUSIONS:** HIV infected women are as other women at risk for congenital anomalies. A full screening program should be offered. HIV infection should not be an absolute contraindication to amniocentesis after appropriate counseling. An effective antiviral therapy should always be given. The risk of transmitting HIV through the procedure seems acceptably low.

#### 45. FRAGILE X CARRIER SCREENING IN THE PRENATAL COUNSELING SETTING IN THE UNITED STATES

**M. Mahoney<sup>2</sup>, A. Cronister<sup>1</sup>, M. DiMaio<sup>2</sup>, A. Donnemfeld<sup>1</sup>, S. Hallam<sup>1</sup>**

<sup>1</sup>Genetic Services and Molecular Diagnostic Laboratory, Genzyme Genetics, Westborough, Massachusetts, USA

<sup>2</sup>Department of Genetics, Yale University School of Medicine, New Haven, Connecticut, USA

**OBJECTIVE:** To document the experience with fragile X carrier screening offered to women seeking prenatal genetic counseling services at two referral centers.

**DESIGN AND METHODS:** 29,103 preconception or pregnant women were offered fragile X screening in 2001-2002, 5454 (19%) at Yale University, an academic medical center, and 23,649 (81%) at sites throughout the United States by Genzyme Genetics, a commercial source of genetic services. Women with a suspected or known family history of fragile X syndrome were not included. Prior to counseling, women received an information sheet that was reviewed during the session. A detailed discussion followed for those who were interested in testing. Those who accepted testing had a blood sample analyzed in the Yale or Genzyme laboratory. FMR-1 allele size was defined as normal (<45 CGG repeats), intermediate (45-54), premutation (55-200) or full mutation (>200). Using the chi-squared statistic, the acceptance rate of screening was analyzed by referral indications for prenatal genetic counseling and, for advanced maternal age women, by whether they elected an invasive procedure (CVS or amniocentesis). The frequencies of the four categories of FMR-1 alleles, the choices of women regarding fragile X prenatal diagnosis and the behavior of premutation and intermediate alleles when transmitted were documented.

**RESULTS:** 7.9% of women accepted fragile X screening (8.0% at Genzyme; 7.2% at Yale). Of 16,008 referred for advanced maternal age  $\geq 35$  yr, 9.8% accepted. For positive multiple marker screening (n=6738), 3.1% accepted; positive family history that did not include suspicion of fragile X (n=4401), 7.5% accepted; abnormal ultrasound (n=1102), 5.8% accepted; possible teratogenic exposure (n=622), 3.4% accepted; and for patient concern (n=232), 40.9% accepted. Among 7906 advanced maternal age women who chose an invasive procedure for fetal karyotype, 11.4% accepted compared to 6.4% who declined a procedure ( $p < .01$ ). There were 16 intermediate alleles (1 in 143 women), 6 premutations (1 in 382) and no full mutation. Three premutation carriers chose fragile X prenatal diagnosis; one premutation was transmitted and remained unchanged (74 repeats). Of seven transmitted intermediate alleles, three had small expansions.

**CONCLUSIONS:** In the United States a low but consequential number of women who seek prenatal diagnosis counseling for reasons unrelated to fragile X will elect fragile X screening. Advanced maternal age, having an invasive procedure or having high concern about fetal disorders are factors significantly associated with a higher acceptance of screening.

#### **46. PRENATAL DIAGNOSIS IN THREE HUNGARIAN FAMILIES AFFECTED BY FACIOSCAPULOHUMERAL DYSTROPHY**

**H. Pikó<sup>1</sup>, J. Balog<sup>1</sup>, Z. Bán<sup>2</sup>, P. Mayer<sup>3</sup>, R. Horváth<sup>4</sup>, V. Karcagi<sup>1</sup>**

<sup>1</sup>National Center of Public Health, Department of Molecular Genetics and Diagnostics, Budapest, <sup>2</sup>Semmelweis University, I. Department of Obstetrics and Gynecology, Budapest, <sup>3</sup>Erzsébet Hospital, Department of Neurology, Hódmezővásárhely, <sup>4</sup>Jahn Ferenc Dél-Pesti Hospital, Department of Neurology, Budapest, HUNGARY

Facioscapulohumeral muscular dystrophy (FSHD) is a neuromuscular disorder with an autosomal dominant inheritance and an incidence of 1/20 000. FSHD is caused by deletion of most copies of the 3,3 kb subtelomeric D4Z4 repeat array on chromosome 4q35. In unaffected individuals, this locus comprises 10-100 tandem repeats. Deletions leaving 1-8 such repeats have been associated with FSHD, for which no candidate gene has been identified yet. FSHD is characterised by weakness and atrophy of the muscles of the face, upper arms and shoulder girdle, but often the disease progresses to affect the peroneal and pelvic girdle muscles. The clinical features of the disease vary widely, both within and between families. In this study we report our experience on the characterisation of the 4q35 molecular rearrangements in familial cases of FSHD. The molecular analysis allowed us to determine the size of the p13E-11 small fragments associated with the disease, to identify subjects at risk of transmitting the FSHD mutation and to perform prenatal diagnoses in three at risk couples, where the affected parent carried a 4q35 *BlnI*-resistant fragment smaller than 38 kb. DNA samples of the family members were analysed by Southern blotting using *EcoRI* and *EcoRI/BlnI* digestion followed by hybridization with the locus specific p13E-11 probe.

DNA was extracted from chorionic villi samples (CVS) at 11–12 weeks of gestation. In all three cases the pregnant women were affected, while the remaining samples investigated were obtained from the healthy spouses. The FSHD status, determined by the presence of the p13E-11 short fragment associated with the at risk haplotype, was detected in one fetus. In the remaining two fetuses the detection of high molecular weight p13E-11 fragments associated with the not at-risk haplotype allowed us to exclude the disease.

In conclusion, we are now able to perform prenatal diagnosis in the majority of at risk pregnancies (95%) with nearly 100% reliability, thus considerably contributing towards the prevention of the disease. Prenatal diagnosis can be offered not only for the affected patient carrying the mutation, but also for the parents of a sporadic patient because of the possibility of germline mosaicism. To the contrary, molecular diagnosis and genetic counselling can be hazardous in those FSHD families in which 4q35 fragments around 38 kb in size are detected. Genetic heterogeneity must be taken into account in these cases and prenatal diagnosis is not feasible.

#### **47. PRENATAL DIAGNOSIS OF FAMILIAL CEREBRO COSTO MANDIBULAR SYNDROME (CCMS)**

**R. Quadrelli, A. Vaglio, A. Diaz, A. Lemes, M. Larvandaburn**

Instituto de Genética Médica, H. Italiano, Montevideo, URUGUAY

**OBJECTIVE:** To present the prenatal diagnosis of a case of familial CCMS, a rare disorder for which different inheritance mechanisms have been described (Frans et al., *Am. J. Med. Genet.* 1996;62:286) and to highlight the importance of making fetal sonographic evaluation guided by previously familial diagnosis.

**DESIGN AND METHODS:** A 33 years old woman, coursing 16 weeks of pregnancy and her husband requested genetic counseling. They had antecedents of CCMS in their first baby who died in the neonatal period. The husband has minor expression of the same syndrome (both diagnosis were performed at our Institute).

After genetic counseling, sonographic evaluation searching features of this syndrome and amniocentesis for cytogenetic study were performed.

**RESULTS:** The sonographic examination of the fetus revealed severe micrognathia, small thorax and short ribs. Cytogenetic study was normal 46,XY. The couple decided interruption of the gestation. Autopsy confirmed the diagnosis.

**CONCLUSIONS:** We present a case of prenatal diagnosis of CCMS by fetal dysmorphology. We highlight the importance of the guided sonographic study as an element for fetal evaluation, when clinic and familial orientating facts are present.

#### **48. MARKEDLY ELEVATED AMNIOTIC FLUID ALPHA-FETOPROTEIN ASSOCIATED WITH FETAL KLIPPEL-TRENAUNAY-WEBER SYNDROME**

**L.P. Shulman<sup>1</sup>, C. Yates<sup>1</sup>, C. Hammond<sup>2</sup>**

Divisions of <sup>1</sup>Reproductive Genetics and <sup>2</sup>General Obstetrics and Gynecology, Department of Obstetrics and Gynecology, Northwestern University, Chicago, Illinois, USA

**OBJECTIVE:** Markedly elevated ( $\geq 10$  MoM) levels of amniotic fluid and serum alpha-fetoprotein (AFP) can be associated with fetal Klippel-Trenaunay-Weber (KTW) syndrome.

**DESIGN AND METHODS:** A woman presented for genetic counseling and amniocentesis after a routine ultrasound at 20 weeks gestation demonstrated a considerably thicker left upper leg in comparison with the right leg. No other abnormalities were identified.

**RESULTS:** An MRI and repeat ultrasound confirmed the initial ultrasound findings: a large cystic mass on the left leg and buttocks without evidence of extension to the spine or anterior abdominal wall. Amniocentesis demonstrated a normal karyotype (46,XX) with a markedly elevated amniotic fluid AFP (25.65 MoM) and a positive acetylcholinesterase. The patient chose to terminate the pregnancy and KTW was confirmed post termination.

**CONCLUSIONS:** Markedly elevated levels of amniotic fluid AFP and, presumably, serum AFP, can be associated with fetal KTW in addition to other fetal conditions. Detailed ultrasound examination of such pregnancies to identify the lesions associated with KTW, along with other structural fetal anomalies, should be a central part of the evaluation of pregnancies characterized by markedly elevated levels of serum and amniotic fluid levels of AFP. In addition, imaging modalities such as MRI may be of value in identifying fetal KTW as well as other fetal structural defects.

#### **49. THE INCIDENCE OF CYSTIC FIBROSIS IN SECOND-TRIMESTER FETUSES WITH HYPERECHOIC BOWEL**

**F. Stipoljev<sup>1</sup>, T. Hafner<sup>1</sup>, J. Sertic<sup>2</sup>, M. Kos<sup>1</sup>, A. Rukavina-Stavljenic<sup>2</sup>**

<sup>1</sup>Department of Obstetrics and Gynecology, Medical School University of Zagreb, Hospital "Sveti Duh",

<sup>2</sup>Clinical Institute of Laboratory Diagnosis, Zagreb University Hospital, Zagreb, CROATIA

**OBJECTIVE:** To determine the significance of fetal hyperechogenic bowel as a specific ultrasonographic marker for the cystic fibrosis in the low-risk pregnant women population.

**DESIGN AND METHODS:** Sixteen fetuses with the echogenicity of bowel of surrounding bone were included in our study. Genomic DNA was obtained from amniocytes and fetal blood. LightCycler-based fluorescent-labeled oligonucleotide melting assay use the fluorimeter to screen DNA samples for the cystic fibrosis mutations. The family history was negative in all cases regarding to cystic fibrosis and bowel disease.

**RESULTS:** The mean gestational age was 19.2 weeks ranging from 17 to 26 weeks of gestation. The screening test for cystic fibrosis transmembrane conductance regulator mutations was performed in all included cases. One fetus was found to be a heterozygote carrier for deltaF508 mutation. The presence of echogenic dilated bowel loops was noted in one fetus homozygous for deltaF508 mutation. The incidence of cystic fibrosis was 6% in our study group. The termination of pregnancy was performed based on the abnormal result of cystic fibrosis mutational analysis.

**CONCLUSIONS:** The appearance of isolated hyperechoic bowel in the second trimester fetuses was found to be associated with a significantly higher risk for cystic fibrosis. The finding of specific ultrasonographic marker indicates the necessity for further prenatal molecular testing, which is of critical importance in low-risk population.

## 50. TEN YEARS EXPERIENCE ON PRENATAL DAGNOSIS OF SPINAL MUSCULAR ATROPHY IN HUNGARIAN FAMILIES. AN OVERVIEW OF 87 CASES

**V. Tarnawa<sup>1</sup>, Á. Herczegfalvi<sup>2</sup>, L. Timár<sup>3</sup>, K. Hajdú<sup>4</sup>, A. Tóth<sup>4</sup>, É. Siska<sup>5</sup>, V. Karcagi<sup>1</sup>**

<sup>1</sup>National Center for Public Health, National Institute of Environmental Health, Dept. of Molecular Genetics and Diagnostics, Budapest

<sup>2</sup>Bethesda Children's Hospital, Dept. of Neurology, Budapest

<sup>3</sup>National Institute of Children's Health, Budapest

<sup>4</sup>National Medical Center, Dept. of Obstetrics and Gynecology, Budapest

<sup>5</sup>National Institute of Psychiatry and Neurology, Dept. of Molecular Neurology, Budapest, HUNGARY

Proximal spinal muscular atrophy (SMA) is the second most frequent autosomal recessive disease. It is caused by the damage of motoneurons of the lower spinal cord, which results in progressive muscle weakness followed by paralysis of all muscles. SMA has been subdivided into three clinical phenotypes (SMA I, II, III) based on severity of symptoms. Homozygous exon deletions in SMN1 gene are responsible for all three types of SMA in 95% of the cases. The SMN1 gene has been mapped to 5q13 region, which contains multiple copies of genes and microsatellite markers. There is a centromeric copy of the SMN gene, SMN2 whose copy number influences clinical severity.

The aim of this presentation is to show the summary of the results of prenatal diagnosis on spinal muscular atrophy which has been performed since 1993 exclusively by our laboratory in Hungary. During these 10 years 303 families were sent with the suspected clinical diagnosis of SMA to our laboratory; out of them 188 were genetically confirmed. As heterozygous state of the parents had to be assumed prenatal diagnosis could be offered in case of new pregnancies for those families where the mutation had been confirmed previously. Up to the present 86 prenatal diagnoses were accomplished in 57 SMA type I. and II. families.

Prenatal diagnosis includes direct mutation analysis and haplotype analysis in order to confirm mutation data and exclude maternal contamination of the CVS DNA. For direct mutation analysis the presence or homozygous absence of exon 7 and exon 8 of SMN1 gene was detected by PCR and RFLP. For haplotype analysis 8-10 microsatellite markers located in the 5q13 region were used by <sup>32</sup>P-labelled PCR.

In summary, 62 healthy and 24 affected fetuses were found among 86 cases. In one case a de novo mutation of the index case was revealed while there was a discrepancy between the haplotype and mutation result of the fetus. Data on confirmation of the rare de novo case will also be presented.

## 51. FIRST TRIMESTER SCREENING FOR TRISOMY 21 AND 18

**R. Wapner**

Department of Obstetrics and Gynecology, Drexel University College of Medicine, Philadelphia, PA, USA

OBJECTIVE: Screening for aneuploid pregnancies is presently performed after 15 weeks of gestation with a sensitivity of 65 percent for a 5 percent false positive rate. Recently, first trimester screening markers have been developed but their performance in clinical practice has not been adequately evaluated.

DESIGN AND METHODS: Pregnant women between 74 and 97 days gestation had screening for trisomies 21 and 18 based on maternal age, free beta human chorionic gonadotrophin, pregnancy associated plasma protein A and nuchal translucency. Risks of 1:270 for trisomy 21 and 1:150 for trisomy 18 were considered screen positive.

RESULTS: Screening was completed in 8,514 patients. The screen identified 85.2 percent (95 percent confidence interval, 73.8 to 93.0) of the 61 Down syndrome pregnancies with a false positive rate of 9.4 percent (95 percent confidence interval, 8.8 to 10.1). At a 5 percent false positive rate, the detection rate was 78.7 percent (95 percent confidence interval, 66.3 to 88.1). Screening identified 90.9 percent (95 percent confidence interval, 58.7 to 99.8) of trisomy 18 fetuses, with a 2 percent false positive rate. In women 35 years old or older 89.8 percent of trisomy 21 fetuses were detected with a false positive rate of 15.2 percent, 100 percent of trisomy 18 fetuses were identified.

CONCLUSION: First trimester screening for trisomy 21 and 18 using maternal age, free beta human chorionic gonadotrophin, pregnancy associated plasma protein A, and fetal nuchal translucency is reliable, and provides superior results early in gestation.

## 52. FIRST TRIMESTER TWO-STEP COMBINED TEST FOR ANEUPLOIDY SCREENING

**A. Fortuny, A. Borrell, E. Casals**

Department of Obstetrics and Gynecology, Hospital Clinic, University of Barcelona, Barcelona, SPAIN

**OBJECTIVE:** To assess the performance of a two-step approach for first trimester aneuploidy screening with the combined test using maternal serum markers and nuchal translucency (NT).

**DESIGN AND METHODS:** Prospective interventional study in 4271 pregnancies. Mean maternal age 31.0 (14-45). Blood sampling at mean gestational age of 9.4 weeks. Ultrasound scan for CRL and NT at mean 12.3 weeks (range 10-14). Maternal serum markers (PAPP-A and fβhCG) were measured in fresh samples by immunofluorometric assay. Values for markers and NT converted into MoM and the Delfia package used for risk estimation. No specific algorithm was applied for trisomy 18. Chorionic villus sampling (CVS) was offered on the same day of the scan if the estimated risk at term was  $\geq 1:250$ . Complete follow-up data for perinatal outcomes is available so far for 3644 pregnancies.

**RESULTS:** In 3644 pregnancies with completed follow-up the overall test (+) rate was 3.9% (2.9% in women < 35 y.o., 6.9% in 35-37 y.o. and 15.4%  $\geq$  in 38 y.o.). The detection rates were 88.8% (8/9) for trisomy 21, 80% (4/5) for trisomy 18 and 75% (3/4) for other aneuploidies. The overall detection rate was 83.3% with a false positive rate of 3.43%.

The estimated ratios of the number of invasive procedures for detected anomaly were 17:1 for trisomy 21 and 9:1 for any aneuploidy. The ratios compare very favorably with previously applied strategies in our Unit as were maternal age  $\geq 35$  using 1<sup>st</sup> trimester CVS (62:1 and 36:1) and second trimester double test screening (82:1 and 47:1).

**CONCLUSIONS:** Our results with the use of the combined test applied in a two-step approach, considering the different optimally performing timings for biochemical and ultrasound markers, has substantially improved as assessed by the detection rates and false positive rates. This contributes to a reduction in unnecessary invasive testing and the expectedly derived fetal loss as well as overall avoidable cost for invasive tests, when compared to our results with the second trimester double test.

## 53. FIRST AND SECOND TRIMESTER SERUM MARKERS: RESULTS OF THE FASTER TRIAL

**J. Canick**

Department of Pathology, Women and Infants Hospital, Brown University, Providence, USA

A requirement in Down syndrome screening is knowledge of the distributions of the markers in both affected and unaffected pregnancies. Not only does the temporal pattern of the marker levels in unaffected pregnancies have to be clearly described, but the pattern of the marker levels in unaffected pregnancies relative to those in affected pregnancies must be delineated as well.

Second trimester marker levels, including AFP, E3, hCG and inhibin A, measured in Down syndrome pregnancies do not change appreciably relative to unaffected levels over the 15-22 week range of gestation. However, the levels of the markers measured in the first trimester are variably informative at different gestational weeks. PAPP-A levels, which are, on average, low in affected pregnancies, are lowest and most informative at the earlier gestational weeks and become non-informative by about 14 weeks, while free beta-hCG levels, which are elevated in affected pregnancies, improve moderately between 10 and 13 weeks. Data on hCG itself and inhibin A relative to their utility as first trimester markers are developing. In the case of nuchal translucency (NT), the only currently used non-serum marker, its effectiveness as a marker appears to decrease between 10 and 13 weeks.

The FASTER Trial was an US National Institutes of Health-funded multicenter study in which 38 000 unselected women agreed to have a first trimester NT sonogram and to provide serum samples in both the first and second trimesters. Screening results were reported only after all samples were collected and analyzed. We have used data from this study to assess the temporal patterns of each of the screening markers discussed above, and in that way estimate their effect on screening performance using different combinations of markers. Results of the FASTER Trial will be presented in the context of other studies already reported in the literature.

## 54. COMPARISON OF DIFFERENT TESTS ON ANTENATAL SCREENING FOR DOWN SYNDROME

### **N. Wald**

Wolfson Institute of Preventive Medicine, Barts and The London School of Medicine and Dentistry, London, UK

Over the past 20 years antenatal screening for Down syndrome has improved substantially. In the early 1980s the only method of screening was to identify women of advanced maternal age and offer them amniocentesis. Subsequently second trimester biochemical markers emerged as being useful in distinguishing affected from unaffected pregnancies and the triple test became the main method of screening. This used maternal age together with the measurement of alpha-fetoprotein (AFP), unconjugated oestriol (uE<sub>3</sub>) and human chorionic gonadotropin (hCG) at about 16-17 weeks of pregnancy. Later inhibin-A was added to form the quadruple test. First trimester ultrasound (nuchal translucency (NT) and serum markers (pregnancy associated plasma protein-A (PAPP-A) and free beta hCG) were also identified. As a result of this development, many different tests became available; with some uncertainty over their relative efficacy on comparing first and second trimester tests. Over the last two years studies have been reported that can compare the different methods of screening in an unbiased way, permitting an objective assessment of the efficacy and safety of the different methods of screening. The studies show that the best screening performance is achieved with the integrated test which involves collecting information in the first trimester (PAPP-A and NT) and the second trimester (AFP, uE<sub>3</sub>, hCG and inhibin-A) of pregnancy but reporting just one screening result after completion of the second stage of the test. The Down syndrome detection rate is about 90% with a false-positive rate of about 2%. The next most effective test is the serum integrated test (PAPP-A in the first trimester and quadruple test markers in the second without NT measurement), followed by the first trimester combined test (free beta-hCG, PAPP-A and NT) or second trimester quadruple test which each have a similar screening performance.

## 55. RAPID DIAGNOSIS OF ANEUPLOIDIES BY QF-PCR. EXPERIENCE ON 5000 SAMPLES.

### **Z. Bán**

I. Department of Obstetrics and Gynecology, Semmelweis University, Budapest, HUNGARY

INTRODUCTION: Multiplex quantitative fluorescent polymerase chain reaction (QF-PCR) analysis of amniotic fluid samples has been shown to be a useful tool in the detection of fetal aneuploidies. It has several advantages over cytogenetic analysis. It is fast (result within a few hours), detects aneuploidies from minute samples and does not require viable cells. QF-PCR has also its limitations as it can not detect some fetal chromosome disorders of clinical importance.

OBJECTIVE: The aims of our study were to test the reliability of amnio-PCR for the prenatal diagnosis of the common aneuploidies (1), to obtain data on the allele distribution of 7 different short tandem repeats (STR) in Hungarian population (2) and to analyze the indications in which amnio-PCR can be applied safely in prenatal diagnosis (3).

DESIGN AND METHODS: 4985 patients (25 twin pregnancies) undergoing amniocentesis we performed both amnio-PCR and karyotyping of the amniotic fluid samples. We compared the amnio-PCR results with those of the conventional cytogenetic study (1). We have analyzed allele distribution of the applied STR markers (2). We compared the occurrence of chromosome disorders which can not be detected by amnio-PCR in cases with different indications of amniocentesis (3).

RESULTS: 98.3% of amnio-PCR results were informative without false-negative and false-positive results. 9 samples (0.16%) were inconclusive because of borderline peak ratios of diallelic results. 126 chromosomal abnormalities of 152 were detectable by amnio-PCR. Amnio-PCR detected all chromosomal disorders in case of abnormal serum marker levels as indication for amniocentesis. In case of structural abnormalities detected by ultrasound, the chromosome disorder was not detectable by amnio-PCR in 23 cases.

CONCLUSIONS: We applied a reliable, simple and cost-effective protocol using 7 STRs for the prenatal diagnosis of the common aneuploidies. In our study on 5000 amniotic fluid samples, all chromosome disorders of clinical significance were detected in case of abnormal serum marker levels. The highest number of chromosome disorders that are not detectable by amnio-PCR was in case of structural abnormalities detected by ultrasound. Prenatal multiplex QF-PCR diagnosis of trisomies 21, 18, 13 and sex chromosome anomalies is reliable, but the indication of the prenatal diagnosis should be considered prior its application.

## 56. ANEUPLOIDY EXCLUSION OR FULL KARYOTYPING: IS FETAL ULTRASOUND USEFUL?

**L.S. Chitty**

University College London Hospitals and the Institute of Child Health, London, UK

Prenatal karyotyping has traditionally been done by culturing amniotic fluid or chorionic villus cells, a procedure taking around two weeks that identifies aneuploidy, sex chromosome anomalies and many other chromosome rearrangements whether balanced or unbalanced. Recently developed molecular methods, FISH or qPCR, will diagnose common aneuploidies and sex chromosome anomalies, but cannot detect most other rearrangements (around 30% of abnormal karyotypes). Although many of these other rearrangements are of little or no clinical significance, many consider that rapid karyotyping should not be offered alone. Retrospective studies have reported that most clinically significant chromosomal rearrangements potentially detectable only by full karyotyping have sonographic abnormalities detectable in the second trimester. Furthermore, as an increased nuchal translucency is associated with structural abnormalities detectable later in pregnancy it may be that unbalanced chromosomal rearrangements will occur more frequently in fetuses with an increased NT. In this paper data supporting the view that full karyotyping, in either the first or second trimesters, could be restricted to fetuses with sonographic abnormalities or an increased NT will be presented. A recent prospective study of amniocenteses has shown that a policy offering rapid karyotyping to all patients, but restricting traditional karyotyping to cases with ultrasound anomalies would reduce the number of full karyotypes requested by 70% while maintaining a 95% detection rate for all clinically important chromosomal rearrangements. A retrospective analysis of 20 000 cases undergoing CVS where nuchal translucency was measured demonstrated that if full karyotyping is restricted to the 30% fetuses with an NT of  $\geq$  median + 1 mm or other sonographic abnormalities, more than 99% of viable chromosomal rearrangements conferring a significant risk of serious handicap will be detected.

In conclusion, fetal ultrasound using nuchal translucency measurement and anomaly scanning can select cases that require full prenatal karyotyping. The balance of costs, benefits and acceptability to health professionals and patients must be explored further and will of course depend on the standard of ultrasound scanning available.

## 57. MOSAICISM IN CVS AND EMBRYOS: LINKING CPM WITH ITS ORIGINS

**J. Wolstenholme**

Institute of Human Genetics, University of Newcastle, UK

OBJECTIVE: Mosaicism is usually considered in the clinical setting, often during prenatal diagnosis, where the next question is 'Is this something I can ignore, or is it something which may be in the fetus?'. Numerous collaborative studies have been devoted to dealing with this practical situation in CVS and amniocentesis. Alternatively, by analysis of what we know about mosaicism throughout pregnancy, it is also possible to consider mosaicism as a dynamic longitudinal process. Inevitably, there are some aspects of mosaicism that are better understood than others, limiting this approach. Our knowledge of mosaicism at the preimplantation stage has been significantly hampered by restricted access to surplus embryos (that may or may not be truly representative), and the technical difficulties of extracting the maximum amount of relevant cytogenetic information from those that are available. Although it has been reassuring to visualise mosaic and non-mosaic abnormalities directly in cleavage-stage embryos, the small numbers of cases studied have meant that with the exception of trisomy 16 and triploidy, it has not proved possible to extract even semi-quantitative data about the incidences of individual chromosome abnormalities.

DESIGN AND METHODS: We have undertaken a large cytogenetic study of over 600 human blastocysts. By combining our data with other recently published findings from cleavage stage embryos, and then linking these to mosaic karyotypes encountered in pregnancy losses, CVS and amniocentesis, we can now start to build a picture of how individual cytogenetic abnormalities behave throughout pregnancy.

## 58. DUCTUS VENOSUS ASSESSMENT FOR TRISOMY 21 SCREENING IN ADDITION TO THE COMBINED TEST

**A. Borrell<sup>1</sup>, H. Cuckle<sup>2</sup>**

<sup>1</sup>Prenatal Diagnosis Unit, Institute of Gynecology, Obstetrics and Neonatology, Hospital Clinic, University of Barcelona Medical School, Barcelona, Catalonia, SPAIN

<sup>2</sup>Reproductive Epidemiology, Leeds Screening Centre, University of Leeds, UK

**OBJECTIVE:** To assess the improvement in screening efficiency when ductus venosus (DV) doppler studies are added to the first-trimester Combined Test.

**DESIGN AND METHODS:** Statistical modeling was used with parameters derived from prospective DV studies and from the published literature. The pulsatility index for veins (PIV) was determined in the fetal DV in 3706 unaffected and 24 trisomies 21 pregnancies at 10-14 weeks gestation, from December 1996 to December 2002. Concurrent nuchal translucency measurement and maternal serum PAPP-A and free- $\beta$  hCG was also measured.

**RESULTS:** The average PIV in trisomy 21 was 1.74 times higher than in unaffected pregnancies. There were no statistically significant correlations between PIV and the other markers. Modeling predicts that for a fixed 5% false-positive rate, the addition of PIV to nuchal translucency alone will increase the detection rate from 76% to 85%, and combined with serum markers (the Combined Test) from 88% to 92%. For a fixed 85% detection rate, the false-positive rate reduced from 15% to 5.2% and from 3.2% to 1.1% respectively.

**CONCLUSIONS:** DV Doppler studies can substantially improve trisomy 21 screening efficiency.

## 59. IS NUCHAL TRANSLUCENCY SCREENING ASSOCIATED WITH DIFFERENT RATES OF INVASIVE TESTING IN AN OLDER OBSTETRIC POPULATION?

**S.T. Chasen, L.B. McCullough, F.A. Chervenak**

Department of Obstetrics and Gynecology, Weill Medical College of Cornell University, New York, USA

**OBJECTIVE:** Our objective was to assess the impact of first-trimester Down syndrome screening on the rates of invasive testing in an older obstetric population.

**METHODS:** Our study population included women 35 or older delivering at our hospital from 1/1/2000-12/31/2002. Records were reviewed to determine whether women underwent nuchal translucency, chorionic villus sampling, and amniocentesis. Chi-square for trend was used to evaluate changes in nuchal translucency, chorionic villus sampling, and amniocentesis rates over six 6-month intervals. Maternal characteristics were compared with Mann-Whitney U test and Fisher's exact test.

**RESULTS:** 4029 women met inclusion criteria. Median age at delivery was 37 years (interquartile range 36-39 years). Rates of nuchal translucency screening increased from 0% to 41.6% over the study interval. Women who underwent nuchal translucency screening when available were older than those who did not (median 37 years, interquartile range 36-40 vs. median 37 years, interquartile range 36-39;  $p = 0.003$ ). A higher proportion of women 40 or older underwent nuchal translucency screening when available than those 35-39 (24.9% vs. 20.4%;  $p = 0.01$ ). Women who underwent nuchal translucency screening were less likely to have chorionic villus sampling compared to those who did not undergo screening, 1.9% vs. 7.1% ( $p < 0.001$ ). Rates of chorionic villus sampling declined over time, while amniocentesis rates remained unchanged. The overall rate of invasive testing declined. Different trends were noted in women 35-39 compared to those 40 and above.

**CONCLUSIONS:** Higher rates of nuchal translucency screening were associated with lower rates of chorionic villus sampling and invasive testing in women 35 and over. The addition of first-trimester screening may lead to reduced rates of invasive testing and fewer losses of normal pregnancies.

## 60. SOFT MARKERS FOR ANEUPLOIDY IN SECOND TRIMESTER OF PREGNANCY

### A. Antsaklis

I. Department of Obstetrics and Gynecology, University of Athens, GREECE

Genetic sonography has been established in the last decade as an important examination, which offers the opportunity to better select candidates for invasive prenatal diagnosis by using a series of sonographic markers that are present more frequently in aneuploid than euploid fetuses. This permits the identification of 60-80 % of affected fetuses and this applies equally well to women whose fetuses are at high and low risk for aneuploidy. Conversely when these sonographic markers are absent, the age specific risk of aneuploidy can be readjusted in women of increased risk of carrying an abnormal fetus because of age. The second trimester sonography includes evaluation of fetal biometry and anatomy for detection of structural anomalies associated with Down Syndrome (DS). Congenital heart disease is the most common anomaly in infants with DS, trisomy 18 and 13. Normal cardiac ultrasonographic examination, theoretically decreases the prior risk for the birth of child with trisomy 21 by 50 % and almost eliminates the risk for trisomy 18 and 13. Non-structural markers (soft markers) included nuchal fold thickness of 6 mm or greater, renal pyelectasis, choroid plexus cyst, hyperechogenic bowel, echogenic intracardiac focus and extremities shortening (humerus and femur). These markers are more common than major or structural abnormalities in fetuses with trisomy 21, are non-specific, they are also present in fetuses without abnormalities, are often transient and they can be readily detected during the second trimester. Sonographic markers were considered isolated when they were not associated with major or other soft marker, but they are often associated with other markers or structural anomalies and the risk of fetal aneuploidy increases with the number of markers present. In addition to the combination of sonographic markers maternal age, and the triple biochemical screening may also add information for the specific risk that a woman has a fetus with DS before a woman makes a decision to undergo amniocentesis. Multivariate analysis of sonographic markers of trisomy 21 has resulted in a detection rate of DS between 75 % and 90% and is present a reasonable alternative to the high risk woman considering invasive testing.

## 61. NUCHAL TRANSLUCENCY AND GESTATIONAL AGE

### H. Aiello<sup>1,2</sup>, L. Otano<sup>1,2</sup>, L. Igarzábal<sup>2,3</sup>, E.C. Gadow<sup>2,3</sup>

<sup>1</sup>Hospital Italiano de Buenos Aires, <sup>2</sup>Grupo de Genética Médica, <sup>3</sup>Centro de Educación Médica e Investigaciones Clínicas-CEMIC, Buenos Aires, ARGENTINA

OBJECTIVE: To evaluate the impact of gestational age on nuchal translucency (NT) measurement.

DESIGN AND METHODS: We examined the NT values in two subset of single pregnancies: 58 fetuses with trisomy 21 and 3269 fetuses with normal karyotype. All values of NT in mm were converted to MoMs for a given crown rump length (CRL). The median and standard deviation of the two groups were calculated and a weighted linear regression of NT in MoM was obtained for each day of gestational age. The NT-MoM values were plotted against gestational age.

RESULTS: The NT values in MoMs of Down syndrome and unaffected fetuses fitted a Gaussian distribution. The NT of trisomy 21 fetuses was 2.24 MoM (mean  $\log_{10}$ : 0.3705; SD  $\log_{10}$ : 0.2103). The NT of unaffected fetuses was 1 MoM (mean  $\log_{10}$ : 0; SD  $\log_{10}$ : 0.1500). The standard deviation of NT in unaffected pregnancies decreased with gestational age: 0.173 at 11weeks, 0.143 at 12-13 weeks, and 0.139 at 14 weeks.

Although not significant ( $p=0.19$ ), the NT MoMs in Down syndrome pregnancies declined with gestational age according to the following formula:  $\log_{10}(\text{NT MoM}) = 1.089 + 0.00813 \text{ gestational age in days}$ . The estimated figures were 2.63 MoMs at 11weeks, 2.36 MoMs at 12 weeks, 2.07 MoMs at 13 weeks, and 1.82 MoMs at 14 weeks, showing a 12% decreased per week.

CONCLUSIONS: A decreasing trend in NT MoMs of affected pregnancies and a decrease in standard deviation on NT values in normal pregnancies were observed. Although these findings did not reach statistical significance, they are consistent with other reports in the literature, and both parameters should be taken into account when calculating an appropriate likelihood ratio for each gestational age.

## **62. ULTRASOUND MINOR AND MAJOR ANOMALIES DETECTED IN FETUSES WITH ANEUPLOIDIES IN SECOND TRIMESTER**

**A. Beke., J.G. Joó, Á Csaba, Cs. Papp. E. Tóth-Pál, Z. Bán, Z. Belics, T. Fekete, E. Barakonyi, Z. Papp**

I. Department of Obstetrics and Gynecology, Semmelweis University, Budapest, HUNGARY

The authors summarize in their report the incidence of the positive ultrasound findings in cases of different chromosome abnormalities. They studied different aneuploidies: trisomies (trisomy 21, trisomy 18, trisomy 13, other trisomies), sex chromosome abnormalities (45,X0 karyotype, 47,XXY karyotype), triploidy and other chromosome abnormalities. They examined in different chromosome abnormalities the cases of subcutaneous oedema: non-immune hydrops, cystic hygroma, in cases of nuchal oedema they measured in the first trimester the nuchal translucency, and in the second trimester the nuchal thickening. From the cerebral malformations they examined ventriculomegaly, choroid plexus cysts, and other cranial malformations. From the cardiac malformations the echogenic intracardiac focus, ventricular/atrioventricular septal defect, and other cardiac (heart and large blood vessels) malformations (coarctatio of the aorta, double outlet right ventricle, hypoplastic left heart syndrome, truncus arteriosus, tetralogy of Fallot) were examined. They also examined pyelectasis, abdominal malformations (omphalocele-exomphalos, duodenal atresia, echogenic bowel), short femur and humerus, and single umbilical artery.

## **63. PRENATAL SONOGRAPHIC MEASUREMENT OF THE FETAL ILIAC ANGLE DURING THE SECOND TRIMESTER OF PREGNANCY**

**Z. Belics, L. Csabay, I. Szabó, A. Beke, T. Fekete, A. Halmos, Z. Papp**

I. Department of Obstetrics and Gynecology, Semmelweis University, Budapest, HUNGARY

**OBJECTIVE:** The purpose of present study was to present our results with sonographic measurement of the fetal iliac angle during the second trimester of the pregnancy. The objective of the study was to determine whether iliac wing angle measurement is a useful sonographic marker for the detection of most common chromosomal aberrations.

**METHODS:** At the Semmelweis University, I. Department of Obstetrics and Gynecology, Budapest, between September 1998 and September 2001, 406 fetal iliac angle measurements were performed in women during the second trimester of their pregnancies. The iliac angle measurements in fetuses with trisomy 21 (n=25), trisomy 18 (n=10) and trisomy 13 (n=5) were compared with iliac angle measurement in fetuses with normal karyotypes (n=333).

**RESULTS:** The mean iliac wing angle in the aneuploid fetuses was as follows: 92.67° in trisomy 21, 79.35° in trisomy 18 and 74° in trisomy 13. The mean iliac wing angle in the healthy fetuses was 70.09°.

**CONCLUSION:** The proven larger iliac wing angle in trisomy 21 can be demonstrated sonographically during the second trimester of the pregnancy, and may be useful sonographical marker in prenatal screening of trisomy 21. We have shown that fetuses with trisomy 18 and 13, on average, have iliac angles only a few degrees larger than healthy fetuses, so the sonographic measurement of the fetal iliac angle can not be used as a ultrasound marker for prenatal screening of trisomy 18 and 13.

#### **64. IS INCREASED AF-AFP ASSOCIATED WITH HIGHER RISKS OF FETAL CHROMOSOMAL OR STRUCTURAL ABNORMALITIES, OR ADVERSE PREGNANCY OUTCOMES?**

**D. Chitayat<sup>1</sup>, T. Huang<sup>2</sup>, A. M. Summers<sup>2</sup>**

<sup>1</sup>The Prenatal Diagnosis and Medical Genetics Program, Mount Sinai Hospital, Toronto, Ontario, CANADA

<sup>2</sup>Genetics Program, North York General Hospital, Toronto, Ontario, CANADA

**OBJECTIVE:** To investigate the associations between increased AF-AFP and the risks of fetal chromosomal and structural abnormalities, and adverse pregnancy outcomes.

**DESIGN AND METHODS:** A retrospective case-control study. The cases (AF-AFP $\geq$ 3.0 MoM) and controls were selected from 12 931 women who were screened in the Ontario MSS program between September 1995 and October 2000, who had an amniocentesis, and measured AF-AFP. Each case was matched with 3 controls for race, screening centre, maternal age, Down syndrome (DS) and neural tube defects (NTD) screening results. The risks of fetal chromosomal, structural abnormalities, and adverse pregnancy outcomes were compared between cases and controls.

**RESULTS:** Ninety one women had AF-AFP $\geq$ 3.0 MoM. They were matched with 225 controls. No control was available for 12 cases, which were excluded from the study. Women with increased AF-AFP had higher risk of having trisomy 18 if cases with an NTD or abdominal wall defects (AWD) were included. When cases with NTD or AWD were excluded, the risks for trisomy 18 and other chromosomal aneuploidies were similar in the case and control groups. As expected, there was a substantially increased risk of CNS abnormalities, NTD and AWD cases. However, the risks of other structural abnormalities were similar in the two groups. Increased AF-AFP is associated with a higher risk of fetal loss but not other obstetric complications.

**CONCLUSIONS:** Patients with isolated high AF-AFP have a higher risk for CNS anomalies including NTD and AWD as expected, and in addition, have an increased risk for fetal loss. They do not have an increased risk for fetal chromosomal anomalies, other structural abnormalities or obstetric complications.

#### **65. MOSAIC TRISOMY (8)(p22p23) IN A FETUS CAUSED BY A SUPERNUMERARY MARKER CHROMOSOME WITHOUT ALPHOID SEQUENCES**

**J.M. de Pater<sup>1</sup>, H.Y. Kroes<sup>1</sup>, M. Dorland<sup>1</sup>, M. Verschuren<sup>1</sup>, C. van Oppen<sup>2</sup>, J.C.M. Albrechts<sup>3</sup>, J.J.M. Engelen<sup>3</sup>**

<sup>1</sup>Department of Biomedical Genetics, University Medical Centre Utrecht, <sup>2</sup>Department of Obstetrics, Gynaecology and Neonatology, University Medical Centre Utrecht, <sup>3</sup>Research Institute Growth and Development, Department of Clinical Genetics, Academic Hospital Maastricht, THE NETHERLANDS

**OBJECTIVE:** Our objective was to characterise a marker chromosome in cultured amniocytes of a foetus with karyotype: mos 47,XX,+mar[3]/46,XX[14].

**DESIGN AND METHODS:** The indication for prenatal cytogenetic analysis of cultured amniocytes was advanced maternal age. Classic banding techniques (GTG- and C-banding) were performed. Microdissection combined with reverse painting was used to disclose the exact origin of the marker; this was confirmed by band specific probes.

**RESULTS:** GTG-banding showed a small marker chromosome in 3 of the 17 colonies analysed. Subsequently, C-banding showed no alphoid sequences, suggesting the presence of a neocentromere. The parent's karyotypes were normal. Elaborate ultrasound analysis of the foetus demonstrated no anomalies. As a result, the parents decided to continue the pregnancy but denied permission for further characterisation of the marker chromosome until after birth. Pregnancy was uneventful. After 41:4 weeks' gestation, the child was born spontaneously with a birth weight of 3410 gram and Apgar scores of 9 and 10 at 1 and 5 min, respectively. No dysmorphisms were seen. Chromosome analysis in peripheral blood after birth demonstrated that the marker chromosome was present in 50% of the lymphocytes. Using microFISH the marker was further characterised and appeared to be derived from chromosome region 8p22-8p23, which was confirmed with FISH using chromosome band specific probes.

**CONCLUSIONS:** The clinical significance of *de novo* marker chromosomes is always a major problem for prenatal counseling. Molecular cytogenetic tools like microFISH are indispensable for characterising markers. Combining the results of GTG- and C-banding analyses and of the microFISH, we conclude that the patient's karyotype is: mos 47,XX,+mar.rev ish der(8)(p22p23)[50]/46,XX[50].

## 66. THE DIAGNOSTIC IMPACT OF REPLACING CONVENTIONAL PRENATAL KARYOTYPING BY SELECTIVE MOLECULAR TESTING

**B. Faas, A. Kooper, P. van den Berg, C. van Ravenswaay, B. Hamel, A. Geurts van Kessel, A. Smits**

Departments of Human Genetics and Gynaecology, University Medical Centre Nijmegen, THE NETHERLANDS

**OBJECTIVE:** Up to now, conventional karyotyping is the gold standard for high-risk pregnant women, as it detects reliably and accurately all numerical and a wide range of structural chromosomal abnormalities present. However, Quantitative Polymerase Chain Reaction (Q-PCR) tests have significant advantages over karyotyping. It requires smaller amounts of cellular material, there is no need to culture the cells, it is efficient and cost effective, less time-consuming and condition-specific in that the test does not provide unexpected results. Q-PCR is suitable for high throughput testing of the five most common aneuploidies (13, 18, 21 and X, Y). In a pilot study (N=200) the Multiplex Ligation-Dependent Probe Amplification (MLPA) Q-PCR test was evaluated as an alternative method for the detection of aneuploidy. All results were concordant with karyotyping. However, it should be emphasized that clinical relevant abnormalities might remain undetected if a Q-PCR test is used, as in such a test only the five most common aneuploidies are included. Therefore, to study the number of such clinical relevant abnormalities, we performed a retrospective study on the results of conventional karyotyping of 5400 amniotic fluid samples obtained from women with advanced maternal age, threshold 36.

**RESULTS:** In total, 116 (2.1%) chromosomal abnormalities were found. Trisomy 13, 18 and 21 were diagnosed in 1.1 %, sex chromosomal abnormalities in 0.2 %, sex chromosomal mosaicisms in 0.2 % and structural balanced abnormalities in 0.4% of the cases. Structural unbalanced chromosomal abnormalities with clinical relevance were encountered in 0.1 % of the cases.

**CONCLUSIONS:** Approximately one clinically relevant chromosomal abnormality per 1000 amniocenteses will remain undetected if Q-PCR testing were to replace karyotyping. These results support the view that implementation of Q-PCR testing may be a suitable alternative for conventional karyotyping.

## 67. ASSOCIATION BETWEEN VERY LOW LEVEL OF PAPP-A IN MATERNAL SERUM AND CONFINED PLACENTAL MOSAICISM FOR CHROMOSOME 3

**N. Ginsberg<sup>1</sup>, G. Tsukerman<sup>2</sup>, M. Sibul<sup>1</sup>, M. Chmura<sup>2</sup>, L. Shuman<sup>1</sup>, Y. Verlinsky<sup>2</sup>**

<sup>1</sup>Department of Obstetrics and Gynecology, Northwestern University, Highland Park, USA

<sup>2</sup>Reproductive Genetics Institute Chicago, Illinois, USA

**OBJECTIVE:** Report the effect of confined placental mosaicism for chromosome 3 on maternal serum PAPP-A.

**DESIGN AND METHODS:** Work up and evaluation of a patient with a PAPP-A value of 0.10 MoM and a nuchal translucency of 0.6 mm at 11 weeks of gestation. Chorionic villi sampling, amniocentesis and cord blood sampling were performed. Ultrasound monitoring with Doppler measurements were performed throughout pregnancy. Placental evaluation was performed both histological and chromosomally were done.

**RESULTS:** Karyotype both direct and culture were all 100% 47 XX+3. Amniocentesis and cord blood sampling were all 46 XX. Level II ultrasound was normal and Doppler measurements remained normal thru out the pregnancy. IUGR of the fetuses was identified and at term a normal 2,285 g female was delivered uneventfully. The newborn was between the 5<sup>th</sup> and 10<sup>th</sup> percentile for weight. The placenta demonstrated multiple chorioangiomas, intervillus fibrin thrombi, small infarct and chronic villitis. Multiple randomly biopsies karyotypically were 47 XX+3.

**CONCLUSIONS:** Confined placental mosaicism for trisomy 3 may cause extremely low levels of PAPP-A and end with the delivery of a normal newborn.

## 68. INCREASING PRENATAL DETECTION RATES OF DOWN SYNDROME IN VICTORIA 1992-2002

**J. Halliday<sup>1,2</sup>, E. Muggli<sup>1</sup>**

<sup>1</sup>Public Health Genetics Unit, Murdoch Childrens Research Institute, Parkville, Victoria, AUSTRALIA

<sup>2</sup>Birth Defects Register (BDR), Victorian Government Department of Human Services, Melbourne, AUSTRALIA

**OBJECTIVE:** To describe the changing patterns of uptake of prenatal diagnostic testing for Down syndrome in Victoria, Australia, over the last ten years with regard to prenatal screening and prenatal detection rates of Down syndrome.

**DESIGN AND METHODS:** Analysis of two well-established datasets: 1. *Prenatal diagnosis*, Public Health Genetics Unit, Murdoch Childrens Research Institute, and 2. *Births and Birth Defects*, Perinatal Data Collection Unit, Victorian Government Department of Human Services, Melbourne, Australia. These relate to approx. 62,000 births/year.

**RESULTS:** The proportion of women aged 37 years and older giving birth has almost doubled from 6.1% in 1992 to 11.2% in 2002 (chi<sup>2</sup> for linear trend =1073.6, p<0.0001). The utilisation of prenatal diagnostic testing in women < 37 years has increased from 2.2% in 1992, to 3.6% in 2002 (chi<sup>2</sup> for linear trend =205.1, p<0.0001), whereas in women aged 37 years and older, uptake of prenatal diagnosis has fallen significantly since 1996, to 42.7% in 2002 (chi<sup>2</sup> for linear trend =642.7, p<0.0001). In 1992, the proportion of all diagnostic tests prompted by an increased risk screening test result was 6.9%, in 1996 and 1998 it was 12.5% and 23.9% respectively. Following this trend, the proportion of diagnostic tests prompted by a screening test result had increased five-fold over the ten-year period to 35.4% in 2002. (chi<sup>2</sup> for linear trend =1272.8, p<0.0001). In 2002 prenatal diagnostic tests have detected the highest proportion of fetal karyotype abnormalities ever recorded. Specifically, 3.41% of all women under the age of 37 years who had a prenatal diagnostic test and 2.44% of all older women ( $\geq 37$ ) who had a prenatal diagnostic test had a fetus with Down syndrome. The proportion of all cases with Down syndrome detected prenatally in the younger age group has increased significantly from 13.5% in 1992 to 69.2% in 2002 (chi<sup>2</sup> for linear trend =63.8, p<0.0001). In women aged  $\geq 37$  years increases in prenatal detection rates were smaller and more fluctuating from 66.7% in 1992 to 82.2% in 2002 (chi<sup>2</sup> for linear trend =3.65, p=0.056).

**CONCLUSIONS:** Over the last ten years, there has been a substantial increase in the proportion of all cases with Down syndrome that are detected prenatally, in particular in younger women where the detection rate has tripled since 1992. Although utilisation of prenatal diagnostic testing in women aged 37 years and over has declined considerably over the past six years, this trend was not accompanied by a concomitant decrease in the proportion of prenatally detected fetuses with Down syndrome in that age group. These findings indicate that current prenatal screening practices are a more effective filter than advanced maternal age alone.

## 69. MATERNAL SERUM SCREENING IN THE SECOND TRIMESTER CAN IDENTIFY THE MAJORITY OF TRIPLOID PREGNANCIES

**T. Huang<sup>1</sup>, E. Alberman<sup>2</sup>, N. Wald<sup>2</sup>, A.M. Summers<sup>1</sup>**

<sup>1</sup>Genetics Program, North York General Hospital, Toronto, Ontario, CANADA

<sup>2</sup>Wolfson Institute of Preventive Medicine. Barts and the London, Queen Mary's School of Medicine and Dentistry, University of London, London, UK

**OBJECTIVE:** To estimate the second trimester prevalence of triploidy, and to investigate the serum marker patterns in these pregnancies.

**DESIGN AND METHODS:** The study included 43 cases of triploidy from 7 second trimester screening programmes in the UK, Canada and the USA. The second trimester prevalence of triploidy was estimated using data from five of the programmes with complete ascertainment of cases. The triploidy detection rates were estimated using the Down syndrome (DS) screening risk cut-off level of  $\geq 1$  in 250 ( $\geq 1$  in 300 for the quadruple test), the trisomy 18 risk cut-off level of  $\geq 1:100$ , and the alphafetoprotein cut-off level of  $\geq 2.5$  MoMs when used as a screening test for open neural tube defects (NTD).

**RESULTS:** The second trimester prevalence of triploidy was 0.34 per 10,000 fetuses. Overall, 58% of triploidies were screen positive for trisomy 18 alone, 9% were positive for NTD alone, and 5% were positive for DS alone. 19% cases had positive results for more than one anomaly.

**CONCLUSIONS:** There is a high detection rate of triploid pregnancies in second trimester screening programme. Although the screening test is not designed to screen for triploidy, 91% of triploid pregnancies were screen positive for at least one anomaly.

## **70. THE ASSOCIATION BETWEEN MARKERS LEVELS AND THE RISK OF SPONTANEOUS FETAL LOSS AS IN A SECOND TRIMESTER MATERNAL SERUM SCREENING POPULATION**

***T. Huang<sup>1</sup>, T. Owolabi<sup>2</sup>, A.M. Summers<sup>1</sup>, C. Meier<sup>1</sup>, P.R. Wyatt<sup>1</sup>***

<sup>1</sup>Genetics Program, North York General Hospital, Toronto, Ontario, CANADA

<sup>2</sup>Maternal Newborn Program, North York General Hospital, Toronto, Ontario, CANADA

OBJECTIVE: To investigate associations between the risk of spontaneous fetal loss and risk estimates assigned by second trimester screening.

DESIGN AND METHODS: The study involved 264,653 women screened in the Ontario Maternal Serum Screening Program between October 1995 and September 2000 with available pregnancy outcomes. Pregnancies associated with fetal chromosomal or structural abnormalities, insulin dependent diabetes mellitus, and multiple pregnancies were excluded. Women were grouped according to risk estimates of Down syndrome, trisomy 18, and neural tube defects respectively. Spontaneous fetal loss rates in each risk group were evaluated after adjusting for losses associated with maternal age and amniocentesis.

RESULTS: Fetal loss rates markedly increased in women with high risk estimates for trisomy 18, neural tube defects, and Down syndrome.

CONCLUSIONS: Risk estimates assigned by triple marker screening may provide an early means of stratifying pregnancies into risk for fetal loss.

## **71. ACCEPTABILITY OF FIRST AND SECOND TRIMESTER SCREENING FOR FETAL DOWN'S SYNDROME – INTERIM RESULTS FROM A DEMONSTRATION TRIAL**

***C.P. Lee<sup>1</sup>, M. Tang<sup>1</sup>, R. Tang<sup>2</sup>, H.Y. Tse<sup>3</sup>, H. Woo<sup>3</sup>, W.K. To<sup>4</sup>, S.F. Wong<sup>5</sup>, K. Wong<sup>6</sup>, Y.H. Lam<sup>1</sup>***

<sup>1</sup>Department of Obstetrics and Gynaecology, Tsan Yuk Hospital, <sup>2</sup>Pamela Youde Nethersole Eastern Hospital, <sup>3</sup>Kwong Wah Hospital, <sup>4</sup>United Christian Hospital, <sup>5</sup>Tuen Mun Hospital, Hong Kong, <sup>6</sup>Centro Hospitalar Conde de S. Januario, Macau, CHINA

OBJECTIVE: To study the acceptability of integrated first and second trimester screening for fetal Down's syndrome in a program which incorporates a choice between first trimester screening by nuchal translucency (NT) alone and integrated screening

DESIGN AND METHODS: 11292 women attending antenatal care before 20 weeks and wanting to have screening for fetal Down's syndrome are recruited. Those over 15 weeks are offered second trimester maternal serum AFP and total hCG (HCG). Those under 15 weeks are offered the choice of integrated screening (IT) (NT between 10 to 15 weeks, AFP, HCG between 16-20 weeks) which has a detection rate of 86% or a first trimester screening (NT) which has a detection rate of 69%, both at 5% false positive, based on a previous study (Lam et al., Prenat. Diagn. 2002;22:730).

RESULTS: 20.6% of the women are above 35 years old. 19.5% are over 15 weeks at booking. Among the 9088 (80.5%) who are under 15 weeks, 461 (5%) chose to have the first trimester NT only while 8627 (95%) chose to have IT. However, 567 (6.6%) of those who chose IT did not complete the screening because of various reasons, including structural abnormalities detected on ultrasound, miscarriages, changing mind about screening before the second trimester biochemical test was due. The overall false positive rate was 4% and the uptake of invasive test after positive screening was 75%. 12 cases of Down's syndrome was detected antenatally among the screened positive cases and 5 additional cases were detected because of structural abnormalities at the ultrasound for NT measurement or before second trimester blood test. Based on the age distribution, the expected number of Down's syndrome cases is 24.

CONCLUSIONS: Most women accepted integrated first and second trimester screening for fetal Down's syndrome when given the choice of a first trimester test with a lower detection rate. Further studies are needed to address the detection rate and also the impact of first and second trimester fetal morphology scans on integrated screening program.

## **72. PRENATAL MOLECULAR CYTOGENETIC DIAGNOSIS OF PARTIAL TRISOMY 1 DUE TO NEOCENTROMERE FORMATION**

**G. Lefort<sup>1</sup>, P. Blanchet<sup>2</sup>, A.-M. Chaze<sup>1</sup>, P. Vago<sup>3</sup>, P. Lochu<sup>4</sup>, H. Lallaoui<sup>5</sup>, A. Pinton<sup>6</sup>, F. Pellestor<sup>7</sup>, P. Sarda<sup>2</sup>, M. Claustres<sup>1</sup>**

Services de Génétique Moléculaire et Chromosomique<sup>1</sup> et Génétique Médicale<sup>2</sup>, CHU Montpellier, Services de Cytogénétique Médicale, CHU<sup>3</sup> et Laboratoire Monier-Chatron<sup>4</sup>, Clermont Ferrand, Cylab, La Rochelle<sup>5</sup>, UMR 898 INRA-ENVT, Toulouse<sup>6</sup>, CNRS UPR 1142, Montpellier<sup>7</sup>, FRANCE

A 40-year-old gravida 2, para 1 woman was referred for amniocentesis at 16 WG for advanced maternal age. Amniotic fluid chromosome studies diagnosed mosaicism for a small supernumerary marker chromosome (SMC) in a male fetus: 47,XY,+mar[13] / 46,XY[8].

The small SMC was negative for CBG and NOR banding. Parental karyotypes were normal. M-FISH studies indicated that the SMC contained chromosome 1 material, and this was confirmed by positive whole chromosome 1 painting. FISH with probes for centromeric chromosome 1 and subtelomeric chromosome 1p and 1q regions was negative.

Microdissection followed by reverse chromosome painting suggested that the marker contained euchromatic proximal 1p and 1q material. In the light of these results fetal prognosis was discussed with the parents who elected to terminate pregnancy despite normal 20 and 24 WG ultrasound scans. Autopsy of the 25 WG fetus showed slight craniofacial dysmorphism, bilateral camptodactyly and rocker bottom feet, but no organ malformation. Cytogenetic studies on amniotic fluid at termination and on tissues at autopsy (skin, lung, heart, kidney,...) confirmed variable mosaicism in all samples. To the best of our knowledge this is the first prenatal report of proximal trisomy 1p/1q due to neocentromere formation.

## **73. CAN RAPID ANEUPLOIDY SCREENING (RAS) REPLACE TRADITIONAL KARYOTYPING FOR WOMEN WITH AMNIOCENTESIS PERFORMED FOR ADVANCED MATERNAL AGE?**

**W.C. Leung, E.T. Lau, M.H.Y. Tang**

Prenatal Diagnostic and Counselling Department, Tsan Yuk Hospital, University of Hong Kong, CHINA

**OBJECTIVE:** To determine the frequency and nature of chromosomal abnormalities that would be missed if RAS (FISH or QF-PCR) were used to replace traditional karyotyping for women with amniocentesis performed for advanced maternal age.

**DESIGN AND METHODS:** We retrospectively reviewed the results of 18,379 amniotic fluid cultures performed for advanced maternal age in a referral centre in Hong Kong from 1997 to 2002. The results were classified as detectable or not detectable by RAS for chromosomes 21, 18, 13, X & Y.

**RESULTS:** 18,228 (99.2%) results were detectable by RAS with 18,060 (98.2%) normal karyotypes and 168 (1.0%) common aneuploidies: T21 (86), T18 (16), T13 (6) & sex chromosomal abnormalities (60). 151 (0.8%) results were not detectable by RAS. 82 (0.4%) of them were balanced translocations and other chromosomal rearrangements of known familial origin with no clinical significance. The remaining 69 (0.4%) were of potential clinical significance: de novo balanced translocations (27), marker chromosomes (10), rare aneuploidies (7), inversions (15), deletions / duplications / others (10).

**CONCLUSIONS:** For every 1,000 amniocentesis performed for advanced maternal age, 4 potentially clinically significant chromosomal abnormalities would be missed if traditional karyotyping were replaced by RAS.

#### **74. A COMPARISON OF AUSTRALIAN AND UK OBSTETRICIANS' AND MIDWIVES' PREFERENCES FOR SCREENING TESTS FOR DOWN SYNDROME: A CONJOINT ANALYSIS STUDY**

**S. Lewis<sup>1</sup>, F. Cullinane<sup>3</sup>, J. Carlin<sup>1,2</sup>, LS. Chitty<sup>5</sup>, A. Bishop<sup>4</sup>, T. Marteau<sup>4</sup>, J. Halliday<sup>1</sup>**

<sup>1</sup>Murdoch Childrens Research Institute, Royal Children's Hospital, <sup>2</sup>CEBU, Royal Children's Hospital, <sup>3</sup>Royal Women's Hospital, Melbourne, AUSTRALIA, <sup>4</sup>Guy's, King's & St Thomas School of Medicine, <sup>5</sup>University College Hospital, London, U.K

**OBJECTIVE:** To describe and compare obstetricians' and midwives' preferences for which screening tests for Down syndrome to offer women.

**DESIGN AND METHODS:** A cross sectional survey using a questionnaire was given to four hundred and twenty two obstetricians and midwives employed at two teaching hospitals: one in Melbourne, Australia and one in London, U.K, (300 Australia, 122 U.K). Nine questions asked the participant to choose between a pair of tests with different attributes i.e. timing, detection rate and risk of miscarriage as a result of subsequent diagnostic tests. Conjoint analysis methodology, which is a rigorous method for eliciting preferences, was undertaken using random effects probit regression in STATA. The main outcome measures were the relative value participants attach to DS screening test attributes when offering a test to women. Marginal rates of substitution of the coefficients and utility scoring of hypothetical tests were performed.

**RESULTS:** 195 (65%) health professionals from Australia and 98 (80%) from the U.K. returned questionnaires of which a total of 269 questionnaires were fully completed and included in the analysis (146 midwives and 29 obstetricians from Australia, 53 midwives and 41 obstetricians from the U.K.). No significant differences were seen between the corresponding Australian and U.K. groups. While obstetricians and midwives shared similar relative values regarding the detection rate of the screening tests they differed in the relative values they attached to the timing of the test and risk associated with the subsequent diagnostic test. Obstetricians were prepared to wait 2.8 weeks for a 1% decrease in risk of procedure-related miscarriage. Midwives would only wait 2.1 weeks. For a test that is a week earlier, obstetricians will accept a reduction in detection rate by 2.8%, but midwives only accept a 1.7% reduction. Marginal rates of substitutions of the coefficients indicate that obstetricians were willing to wait longer for a test that has a lower risk of miscarriage as compared with midwives.

**CONCLUSIONS:** Although the health care systems in the U.K. and Australia are different with regards to uptake of prenatal screening tests, the health professional groups in both countries place almost identical importance on test attributes. Obstetricians place a higher value on the relative safety of prenatal tests than do midwives when considering optimal tests to offer women. The causes of these differences await further research.

## 75. PAPP-A / PROMBP IN PRENATAL SCREENING OF SEVERE FETAL DEVELOPMENT DISORDERS AND POSTNATAL DETECTION OF ACUTE CORONARY DISEASE

**M. Macek<sup>1</sup>, P. Hájek<sup>2</sup>, S. Vilimová<sup>1</sup>, P. Potuníková<sup>1</sup>, M. Simandlová<sup>1</sup>, R. Vlk<sup>3</sup>, M. Havlovicová<sup>1</sup>, M. Hladíková<sup>4</sup>**

<sup>1</sup>Institute of Biology and Medical Genetics, <sup>2</sup>Dept. of Invasive Cardiology, <sup>3</sup>Dept. of Gynecology & Obstetrics, <sup>4</sup>Institute of Medical Informatics, all 2. School of Medicine, Charles University, Prague, CZECH REPUBLIC

**OBJECTIVE:** The efficiency of PAPP-A/PROMBP complex with beta-hCG maternal serum screening for early prenatal detection of severe chromosomal anomalies and prenatal disorders was evaluated by the degree of analyte deviation from norm. PAPP-A/PROMBP levels were also studied in various types of acute coronary disease (CAD).

**DESIGN AND METHODS:** These analytes were examined by Delphia and Kryptor technologies in 1,494 women during the 11<sup>th</sup>–14<sup>th</sup> weeks of gestation. Control group I. had both analytes >0.6–<1.9 MoM. Group II. included pregnancies with 0.5-0.6 and 1.9-2.0 MoM values of one or both analytes. Group III. had abnormal only one analyte with <0.5 and/or >2.0 MoM, while Group IV. had abnormal levels of both analytes. The screening efficiency was evaluated by the proportion of pregnancies with spontaneous abortions, prematurity and preeclampsia and/or severe congenital anomalies in Groups I.-IV. Levels of PAPP-A / PROMBP complex were evaluated in adult controls without- and in patients with various types of CAD.

**RESULTS:** No severe chromosomal anomalies were found in 570 women from Group I. In Group II. only mosaic 45,X/46,XX (1/127) was disclosed. One case of trisomy 21 and FraX-A syndrome were detected in 414 women from Group III. The triploidy (1x), trisomy 13 (3x), trisomy 21 (2x), 45,X/46,XX (1x) were discovered in 7 / 83 (8.43 %) pregnancies in Group IV. The detection rate of chromosomal aberrations is significantly increased compared to Group I. ( $p < 0.001$ ) and from the detection rate of trisomy 21 from 2<sup>nd</sup> trimester screening (2/400–0.5%;  $p < 0.001$ ). The risk of severe congenital anomalies was not increased in Group IV. contrary to the increased risk of severe disorders of pregnancy (2/83–2.4 %;  $p < 0.001$ ). Patients with unstable angina pectoris and different types of acute myocardial infarction are characterized by significantly increased levels of PAPP-A/PROMBP complex compared to controls and patients with stable CAD with specificity and sensitivity significantly higher than troponin I and CRP.

**CONCLUSIONS:** The increased risk of severe disorders of pregnancy and aneuploidies corresponds to the degree of the deviation of analytes from controls. It reflects severe impairment of the placental function. The increased levels in different types of CAD might be linked to the coronary vessels remodelling / repair. The common basis of the diagnostic impact of the PAPP-A / PROMBP complex might be in the mutual interaction of these proteins in local tissue IGF-1 availability for cell growth, glucose transport, amino acids and in blocking the cytotoxic effect of MBP.

## **76. FIRST TRIMESTER SCREENING FOR DOWN SYNDROME IN TWIN PREGNANCIES USING EITHER NUCHAL TRANSLUCENCY OR THE COMBINED TEST**

**M.A. Martínez, A. Goncé, A. Borrell, I. Mercadé, E. Casals, A. Fortuny, V. Cararach**

Institut Clínic de Ginecologia, Obstetrícia i Neonatologia, Hospital Clínic, Barcelona, SPAIN

**OBJECTIVE:** To assess the effectiveness of the addition of first trimester biochemistry to nuchal translucency measurement to screen for Down syndrome in twin pregnancies.

**DESIGN AND METHODS:** Ninety-four twin pregnancies were studied in our center, being maternal serum free-b-hCG and PAPP-A determined at 8-12 weeks and fetal nuchal translucency at 11-14 weeks. An individual risk was estimated for each of the fetuses using nuchal translucency (+ maternal age) and the Combined Test (maternal age + nuchal translucency + first trimester biochemistry). Pregnant women at high risk for Down syndrome ( $>1/250$ ) were offered an invasive diagnostic procedure. In the first 70 pregnancies, the risk derived from nuchal translucency and maternal age was the single criteria used in clinical practice. After a preliminary analysis of the results, the Combined Test was considered to be a better screening method.

**RESULTS:** Using nuchal translucency, all the fetuses with Down syndrome were detected (three fetuses in two pregnancies) with a 9.8% false-positive rate (9/91). With the addition of biochemistry the detection rate remained unchanged (3/3) but the false-positive rate decreased to 4.3% (4/91).

**CONCLUSION:** The shift from nuchal translucency to the Combined Test in the screening for Down syndrome in twin pregnancies appeared to substantially decrease the false-positive rate maintaining an optimal detection rate.

## **77. TO WHAT EXTENT CAN THE MLPA TECHNIQUE REPLACE STANDARD CHROMOSOME ANALYSIS?**

**A.W.M. Nieuwint<sup>1</sup>, J.M. de Pater<sup>2</sup>, K. Madan<sup>1</sup>**

<sup>1</sup> Department of Clinical Genetics and Human Genetics, VU Medical Center, Amsterdam

<sup>2</sup> Department of Medical Genetics, University Medical Center, Utrecht

A collaborative study of all the clinical cytogenetics laboratories in THE NETHERLANDS

**OBJECTIVE:** Multiplex Ligation-dependent Probe Amplification (MLPA) has proved to be a reliable, cheap and quick technique for the detection of the most common aneuploidies. Our objective is to determine how many phenotypically abnormal fetuses would have been missed if pregnant women referred for advanced maternal age would have had the MLPA test instead of karyotyping.

**DESIGN AND METHOD:** We collected data on the chromosome abnormalities found among 65,189 prenatal diagnoses in the advanced maternal age group over a period of several years in the Netherlands. We considered the different types of chromosome abnormalities found in this group and estimated the number that would have been missed by the MLPA test.

**RESULTS:** MLPA would have detected 1045 of all 1842 abnormalities found by karyotyping (56.7%). The remaining 797 abnormalities would have been missed by MLPA. Of these, 16.8% (134/797) was associated with phenotypic abnormalities, i.e. 7.3% of all abnormalities (134/1842). The false negative rate for "clinically relevant" abnormalities is  $134/65189 = 0.2\%$ .

**CONCLUSION:** These results, as well as other factors (such as economical, ethical and psychosocial), should be taken into consideration before any decision on the implementation of the MLPA technique to replace standard chromosome analysis is taken.

## **78. UNEXPECTED MALFORMATIONS IN A FEMALE FETUS WITH A DELETION Xp: DEMONSTRATION OF A CRYPTIC TRANSLOCATION BY MLPA**

**D. Olde Weghuis, C. van Ravenswaal, N. de Leeuw, J. Creemers, B. de Vries, E. Sistermans**

Department of Human Genetics and Gynecology, University Medical Centre, Nijmegen, THE NETHERLANDS

CASE: A woman, gestational age 31 weeks, was referred to the department of Gynecology because of fetal growth retardation. On fetal ultrasound a heart defect and double bubble, indicating duodenal atresia, were seen.

METHODS: Amniocentesis was performed and the cells were cultured for routine cytogenetic analyses and FISH. Directly after birth, EDTA-blood from the umbilical cord was sampled for Multiplex Ligation-dependant Probe Amplification (MLPA) of the subtelomeric regions. Blood from both parents was collected for routine cytogenetic analysis.

RESULTS: Cytogenetic analyses was performed on cultured amniocytes and an unbalanced female karyotype with deletion of the short arm of one of the X-chromosomes was found: 46,X,del(X)(p22,2). Subsequent FISH analysis with a paint specific for Xp showed fluorescence on the entire normal and aberrant Xp. Both parents had normal karyotypes. At 39 weeks of gestation a girl was born with a birth weight of 1496 grams. Duodenal atresia and heart malformation were confirmed and in addition she appeared to have a cleft palate with micrognathia, ectrodactyly of the right hand, corpus callosum aplasia and hypoplastic kidneys. During the neonatal period a central diabetes insipidus became apparent. MLPA analysis on postnatally obtained blood showed a duplication of 19p. This duplication was confirmed by FISH with a subtelomeric probe for 19p, and the extra signal appeared to be located on the short arm of the aberrant X-chromosome.

CONCLUSIONS: We describe a girl with multiple anomalies and a de novo cryptic unbalanced translocation: 46,X,der(X)t(X;19)(p22,2;p13,3), resulting in a partial deletion of Xp and a partial duplication of 19p. However, deletions of Xp usually only give rise to mild Turner stigmata and therefore the phenotype of our patient is more likely to be caused by duplication of 19p. Duplications of 19p are extremely rare. This is most probably due to the gene richness of chromosome 19 and of the difficulty to demonstrate small duplications of this negatively staining chromosome. The two cases described before had some features in common with our patient: growth retardation, micrognathia, heart defect and hypoplastic kidneys. The severe phenotype in our patient is remarkable since a skewed X-inactivation in favor of the normal X-chromosome would be expected. Currently we are performing X-inactivation studies in our patient.

## **79. RECURRENT TRISOMY 21 AND UNIPARENTAL DISOMY 21 IN ONE FAMILY**

**J. Oroszné Nagy, Z. Bán, Gy.R. Nagy, Z. Papp**

I. Department of Obstetrics and Gynaecology, Semmelweis University, Budapest, HUNGARY

OBJECTIVE: A 32-year-old pregnant woman was referred to our genetic counselling because of recurrent trisomy 21 in the family. Karyotype 47,XY+21 of the fetus was found at analysis of amniotic fluid cell culture.

METHODS: Karyotyping and molecular analysis was undertaken on the fetal and parental samples to determine the origin of the extra chromosome 21.

RESULTS: Both parents had normal blood karyotype. Microsatellite marker analysis showed maternal origin of the fetal extra chromosome 21. As the mother showed homozygosity for all investigated markers on chromosome 21, we also tested her family. We detected the same homozygosity in some family members, which was consistent with isodisomy of the chromosome 21 caused by uniparental disomy (UPD).

CONCLUSION: Here we report on a family, in which multiple aneuploid conceptions occurred with trisomy 21 and molecular analysis showed, that the euploidy of the investigated healthy family members is due to UPD21. This observation stresses the importance of prenatal cytogenetic and molecular analysis in case of parental UPD.

## 80. PERFORMANCE OF FIRST TRIMESTER NASAL BONE EVALUATION AS A MARKER FOR DOWN SYNDROME

**L. Otano<sup>1;2</sup>, H. Aiello<sup>1;2</sup>, L. Igarzábal<sup>2;3</sup>, T. Matayoshi<sup>2;3</sup>, E.C. Gadow<sup>2;3</sup>**

<sup>1</sup>Hospital Italiano de Buenos Aires, <sup>2</sup>Grupo de Genética Médica, <sup>3</sup>CEMIC, Buenos Aires, ARGENTINA

**OBJECTIVE:** to evaluate the performance of routine ultrasound examination of the fetal nasal bone (NB) between 11 and 14 weeks of gestation as a marker for trisomy 21 .

**DESIGN AND METHODS:** Between October 2001 and December 2003, we prospectively examined all fetuses from single pregnancies for absence or presence of NB during routine scan before chorionic villous sampling at 11 to 14 weeks of pregnancy. For the purpose of the present study, the groups of normal karyotypes (n=1360) and trisomy 21 fetuses (n=27) were included. Cases where a successful view of the fetal profile was not possible were classified as "not successful". Maternal age, CRL, and nuchal translucency (NT) were also recorded. Detection rate (DR), false positive rate (FPR), positive and negative likelihood ratios were calculated.

**RESULTS:** The median maternal age was 36 years (range 23-42), and the median CRL was 65,2 mm (range 45-85). In 38/1387 cases (2,7%) the examination was categorized as "not successful", 36 of which had normal karyotype and 2 trisomy 21. The rate of "not successful" evaluations decrease from 4% in the first 300 cases to a current 1%. From the 1349 cases with a successful evaluation, nasal bone was absent in 11 out of 1324 fetuses with normal karyotype (FPR: 0,83 %), and in 8 out of 25 fetuses with trisomy 21 (DR: 32 %). The positive likelihood ratio for an absent nasal bone was 38,5, and the negative likelihood ratio was 0,69. In fetuses with normal karyotype, NT was significantly higher in the 11 fetuses with absent NB: 1,7 MoM.

**CONCLUSIONS:** Between 11 and 14 weeks of pregnancy, NB was absent in 1 every 3 trisomy 21 fetuses and in less than 1% of non affected. Absence of NB showed an association with increased NT in normal fetuses. Evaluation of NB in first trimester needs a significant training period, even for experienced operators. Before introducing this marker into the clinical practice, more information from large prospective studies in low risk population is needed.

## 81. CYTOGENETICS AND PATHOLOGY IN THE EVALUATION OF SPONTANEOUS ABORTION

**M. M. Perez Iribar<sup>1</sup>, V. Cusi<sup>2</sup>, A. Aguayo<sup>1</sup>, T. Zabala<sup>1</sup>, A. Vela<sup>3</sup>**

<sup>1</sup>Secció Genética, <sup>2</sup>Servei Anatomia Patològica, <sup>3</sup>Servei Obstetricia i Ginecologia, HSJD. Esplugues, Barcelona, SPAIN

**OBJECTIVE:** The objective of this study was to assess the routinely karyotype and pathology of spontaneous abortion material in our hospital during the last five years.

**DESIGN AND METHODS:** We reviewed the records of our laboratory from March 1998 to May 2003 of spontaneous losses tissues between 5 and 40 weeks' gestation. Depending on the cases we cultured Achilles' tendon, foetal skin or cartilage samples. We have used the long-term culture.

**RESULTS:** We classified the cases according the clinical indication, maternal age and week's gestation and the most frequent was missed abortion. There were 204 submitted samples : 89 (43.6%) failed to grow in culture, 28 (13.7%) were contaminated and 17 (8.3%) weren't suitable for culture. We obtained cytogenetic results of 40 samples (34.3%). Overall, sixteen cases (23%) revealed an abnormal karyotype: five trisomies, four polyploidy, two 45,X monosomy, one structural abnormality, and four mosaics. We had had seven molar gestations. Partial hydatiform moles represent a non-invasive placental disease that is characterized by two populations of chorionic villi, one of normal size and the other grossly hydropic with trophoblastic proliferation. The diagnosis of partial molar pregnancy was correct.

**CONCLUSIONS:** Our cytogenetic results are similar to other series. The output of the technique was low; the reason was the reception of the samples. The complementation of cytogenetic and pathology studies is a good tool to characterize spontaneous losses.

## 82. THE 11-14 WEEK SCAN. SCREENING FOR STRUCTURAL AND CHROMOSOMAL FETAL DEFECTS

**M. Pérez, A. Borrell, M.T. Farré, V. Borobio, F. Figueras, B. Puerto, A. Fortuny, V. Cararach**

Prenatal diagnosis unit, Institute of Gynecology, Obstetrics and Neonatology, Hospital Clinic, University of Barcelona Medical School, Barcelona, Catalonia, SPAIN

**OBJECTIVE:** To assess the effectiveness of the first trimester scan, performed at 11-14 weeks in the detection of congenital defects (either structural or chromosomal)

**DESIGN AND METHODS:** From 1998 to 2002 a first trimester scan (early anomaly scan and NT measurement) was performed in 4365 unselected pregnancies at 11 to 14 weeks. High risk pregnancies were excluded (even those of maternal age  $\geq 38$ y). Risk for T21 was estimated by means of the Combined Test. A universal routine anomaly scan at 20 weeks and a third trimester scan for growth assessment were performed in all of the pregnancies in our centre or elsewhere. Perinatal follow-up was achieved in 97 % of all of these pregnancies.

**RESULTS:** Among the studied pregnancies: 187 (4.3%) structural defects with normal karyotype were found, 68 (1.6%) of which had a major structural defect.

In 18 of these studied pregnancies (0.4%) a chromosomal anomalies were detected.

The gestational age at which these major structural defects were detected was: 31% at 11-14 w; 29% at 15-26 w; 9% at 31-38 w and 32% of them were detected postnatally

The combined test was able to detect 89% of T21; 80% of T18-13; 75% other chromosomal anomalies.; having a 3.8 % false positives.

**CONCLUSIONS:** 1) Around one third of major structural defects were detected at the 11-14 week scan. 2) The most frequently detected anomalies by systems were those of CNS, skeleton, the digestive tract and hydrops. 3) Diaphragmatic hernia was one of the most frequently found structural anomaly. 4) Up to 90% of chromosomal anomalies were detected with the combined test.

## 83. FIRST TRIMESTER SCREENING FOR DOWN SYNDROME IN PRIVATE PRACTICE COMBINING BIOCHEMICAL MARKERS AND NUCHAL TRANSLUCENCY MEASUREMENTS. RESULTS OF 11000 CONSECUTIVE PREGNANCIES

**G. Pescia<sup>1</sup>, P.-J. Ditesheim<sup>2</sup>, Ch. Faway<sup>1</sup>, H. Nguyen The<sup>3</sup>, D. Schmid<sup>4</sup>, P.-A. Brioschi<sup>2</sup>**

<sup>1</sup>Department of Medical Genetics, AMS Laboratories; Services of Gynaecology-Obstetrics <sup>2</sup>Hospital Nyon, <sup>4</sup>Hospital Morges, <sup>3</sup>Pré-du-Marché, Lausanne, SWITZERLAND

**OBJECTIVE:** to demonstrate the feasibility of first trimester combined screening for Down syndrome (DS) and to evaluate the performance of screening in private practice.

**DESIGN AND METHODS:** Between September 1999 and February 2004 first trimester combined screening was offered to 11000 women attending prenatal care with a private obstetrician. All patients had dating scan together with nuchal translucency (NT) measurements. Information about the screening was individually provided by the obstetrician and informed consent was obtained at the same time. All 65 participant obstetricians received training for NT measurements either at the UK Foundation for Fetal Medicine or by FMF trained sonographers. All blood samples were analysed by the same laboratory, levels of PAPP-A and FhCG were measured using *Kryptor*. The laboratory established his own medians and subscribed monthly to external quality control (NEQAS). The risk of Down syndrome (DS) was calculated using the *CISline* software which take into account maternal characteristics (age, ethnicity, weight), levels of PAPP-A and FhCG and measurements of NT. Positive screening was defined as a risk of DS  $\geq 1/380$  at term. Positive cases underwent prenatal diagnosis (CVS or amniocentesis). Genetic analyses include full karyotype and, if needed, direct methods for rapid detection of aneuploidies (FISH, QF-PCR). Screening performance was estimated by the detection rate (DR), the false-positive rate (FPR) and the odds of being affected given a positive result (OAPR). **RESULTS:** Mean maternal age of the 11000 women was 30.5 years, there were 124 twins pregnancies. Mean gestational age at time of screening was 86 days. NT measurements were obtained in 10102 pregnancies (91.8%) and, for the remainder (898 pregnancies), risk calculation was based only on maternal age and biochemical results. Cytogenetic analyses identified 22 cases of DS and 19 other chromosome anomalies. Two patients with negative results of screening delivered a child with DS (false negative). FPR was 6.9% (6.6% with NT, 9.8 without NT), DR was 91.6% (22/24) and OAPR 1:34.

**CONCLUSIONS:** our results confirm the superiority of first trimester screening over other screening strategies and illustrate its feasibility and efficiency in private practice.

#### **84. MONITORING OF GEOGRAPHICAL INEQUALITIES OF DOWN SYNDROME OCCURRENCE IN HUNGARY AND ITS APPLICATION IN PRENATAL SCREENING RELATED PROBLEMS IDENTIFICATION**

***J. Sándor<sup>1</sup>, M. Szunyogh<sup>1</sup>, J. Métneki<sup>1</sup>, Cs. Siffel<sup>1,2</sup>***

<sup>1</sup>Bela Johan National Center for Epidemiology, Department of Human Genetics and Teratology, Budapest, HUNGARY,

<sup>2</sup>National Center on Birth Defects and Developmental Disabilities, Centers for Disease Control and Prevention, Atlanta, Georgia, USA

**OBJECTIVE:** The Hungarian Congenital Malformation Registry belongs to the European registries processing the highest case numbers. Because the notifications of congenital anomalies are geographically coded according to the living place of mothers, the tools of geographical epidemiology can be applied. This opportunity was utilised in processing the Hungarian data on congenital anomalies, in order to improve the effectivity of the register in monitoring for prenatal screening related malpractice.

**DESIGN AND METHODS:** Records from 1980 to 2001 in the database of Hungarian register were coded geographically by zip codes. The Down syndromes were selected and different district level prevalences (for live births and for the whole population of still and live births) were calculated, which were applied as the indicators for malpractice in screening. The district specific indicators were computed for screened and non-screened populations separately, taking into account the pregnancy outcome.

**RESULTS:** The register detected 3415 Down syndrome (0,13% of still and live births) in the study period. High proportion of cases (83%) was live birth. The district level risk of occurrence varied between 64% and 173% of the national average. The range for live births was a bit narrower: 71% - 156%. The districts with high Down syndrome prevalence for live birth to mother above 35 years have been identified and mapped.

**CONCLUSIONS:** It was revealed that the screening practice shows high spatial variability in Hungary. Therefore, the prevention of Down syndrome could be improved by facilitating the use of recommended protocols, at least, in the catchment areas of centers with unfavourable data.

## **85. EXPERIENCE WITH FIRST TRIMESTER SCREENING IN THE NETHERLANDS**

***P.C.J.I. Schielen, L.H. Elvers, J.G. Loeber***

National Institute of Public Health and the Environment, Diagnostic Laboratory for Infectious Diseases and Perinatal Screening (LIS), THE NETHERLANDS

**OBJECTIVE:** In august 2002, we embarked on first trimester screening for Down syndrome by assignment of the Health Care Inspectorate of the Dutch Ministry of Health, Welfare, and Sports. Currently, about thirty Dutch hospitals participate. Due to the Dutch governmental policy on Down syndrome and NBD screening, the Dutch screening program incorporates especially the pregnant women over 34 years of age. Here, the first results of this program are presented, with special emphasis on logistic organisation.

**METHODOLOGY:** Sera were sent to our laboratory by hospital gynaecologists, stored at 4 °C until analysis and analysed for pregnancy-associated plasma protein A and the free beta subunit of human chorion gonadotropin by a commercially purchased assay. Laboratory measurements were transformed into risk estimations by commercially available risk calculation software. A measurement of nuchal translucency (NT), provided by the hospital gynaecologists, was incorporated in the risk calculation. Pregnant women were requested to send in the outcome of pregnancies.

**RESULTS:** Currently, the number of 1<sup>st</sup> trimester tests in our laboratory (forecast for 2004: 10000 samples) exceeds the number of triple tests by far. Part of the hospitals chose a logistic approach where a blood sample is taken at 10-11 weeks of gestation and an NT measurement is performed at about 12 weeks. Other hospitals appear to perform blood sampling and NT concurrently, at about 12 weeks of gestation. The multiple of the median values for the 1<sup>st</sup> trimester test parameters were within the demands (log-normality, amount of bias) of the risk estimation, with a possible exception for NT measurements. Preliminary analysis of the data on the outcome of pregnancies shows that the false positive rate is about 5% and the detection rate is about 80%.

**CONCLUSION:** Within a short period of time, the first trimester screening based on measurement of PAPP-A, free beta hCG and NT for Down syndrome has become widely used for Down syndrome risk estimation. Apparently, a number of logistic scenarios can be facilitated by our laboratory. Preliminary data show that FPR and DR of the program are in agreement with those published in the literature.

## **86. STRUCTURAL CHROMOSOMAL ABERRATIONS IN RELATION TO INCREASED NUCHAL TRANSLUCENCY**

***S. Snijder, A.C. Knegt, M.A. Muller, M. Verjaal, C.M. Bilardo***

Department of Clinical Genetics, Department of Obstetrics and Gynaecology, Academic Medical Centre, Amsterdam, THE NETHERLANDS

**OBJECTIVE:** Increased nuchal translucency (NT) is associated with chromosomal anomalies, especially aneuploidies. This study focuses on the correlation between increased NT and structural chromosomal aberrations.

**DESIGN AND METHODS:** All women referred to our centre for NT screening between January 2000 and December 2003 were included in this study (approximately 5000 women, exact data will be presented at the conference). NT measurements were matched with cytogenetic results from chorion villus sampling or amniocentesis. Chromosomal aberrations in fetuses with a NT thickness above 3.5 mm (99<sup>th</sup> centile) or within normal ranges were compared.

**RESULTS:** Besides numerical aneuploidies and balanced (familial) structural chromosomal aberrations, 14 cases of structural chromosomal aberrations were observed. In 7 of these fetuses (50%) the NT thickness was more than 3.5 mm. The type of structural chromosomal aberration and outcome of pregnancy found in fetuses with a NT thickness above 3.5 mm was compared to the group of fetuses with a NT measurement within normal ranges.

**CONCLUSIONS:** This study suggests a correlation between increased nuchal translucency thickness and unbalanced structural chromosomal aberrations. For adequate screening for such aberrations we recommend high resolution banding in long term culture cells.

## **87. CAN TRISOMY 21 SELECTIVE AMNIO-PCR BE PART OF DOWN SYNDROME SCREENING?**

**D. Stejskal, M. Brouckova, M. Brestak, M. Louckova**

Gennet CZ, Prague, CZECH REPUBLIC

OBJECTIVE: To model efficiency of trisomy 21 specific Amnio-PCR at Down syndrome (DS) screening positive mothers under 35 years.

DESIGN AND METHODS: 11562 amniocenteses with 360 chromosomal aberrations diagnosed between 1993 –2003 were analyzed: 57% procedures and 35% (n=128) aberrations were indicated by positive/atypical multi-marker screening mostly at mothers under 35 years. DS risk was higher than 1/250 at 72% (n=92) aberrations in positive/atypical screening group: 50 (54.4%) trisomies 21, five (5.5%) serious aberrations, four (4.3%) X/Y aneuploidies and six (6.5%) markers. 27 cases (29.3%) were balanced aberrations or X/Y mosaics.

RESULTS: If all DS screening positive younger mothers had been offered selective trisomy 21 Amnio-PCR, all 50 cases (1:90) of DS would have been detected. Only 15 significant different aberrations (1: 312) would have been missed and result corrected after full karyotype. The rest of mothers (98.6 %) would have obtained favorable, correct and definite information.

CONCLUSIONS: Chromosome 21 specific Amnio-PCR can quickly reveal stress of false DS screening positive mothers particularly in younger age. Such specific procedure in specific indication could be positively considered by health planners.

## **88. EVALUATION OF MULTIPLEX LIGATION-DEPENDENT PROBE AMPLIFICATION AS A MEANS TO RAPIDLY DETECT UNBALANCED ABNORMALITIES OF ALL CHROMOSOMES IN PRENATAL CYTOGENETIC ANALYSIS**

**R.F. Suijkerbuijk, B. Sikkema-Raddatz, T. Dijkhuizen, J. Dijkhuis, A.Y. van der Veen, K. Bouman, K.B.J. Gerssen-Schoorl**

Department of Clinical Genetics, University Hospital, Groningen, THE NETHERLANDS

OBJECTIVE: The usability and reliability of multiplex ligation-dependent probe amplification (MLPA) as a means to rapidly detect unbalanced chromosomal abnormalities for all chromosomes in prenatal cytogenetics has been evaluated.

DESIGN AND METHODS: MLPA is a novel, PCR-based, method for relative quantification of up to 40 DNA sequences by means of a simple, single-tube assay (Schouten et al., *Nucleic Acids Res.* 2002;30:12, e57). By employing MLPA for all chromosomal ends many of these unbalanced abnormalities, including those involving subtelomeric regions only, may be detected. Currently, we are evaluating MLPA for all chromosomal telomeres by retrospectively testing a panel of over 50 (normal and abnormal) randomised DNA samples. Preceding this test, a series of over 20 normal DNA samples was examined in order to statistically establish confidence intervals of 95% and 99% reliability, serving as thresholds for genomic imbalances for each MLPA probe used. Results of our evaluation will be compared to those of cytogenetic, fluorescent in situ hybridisation (FISH) and/or microarray comparative genomic hybridisation (CGH) analyses.

RESULTS: Our first results of the MLPA evaluation show that for all test samples examined so far (N = 10) the abnormal samples (N = 7) and their aberrations were easily identified and reliably detected, respectively, as confirmed by (molecular) cytogenetics. Most striking examples being two, cytogenetically unsolved, subtelomeric unbalanced rearrangements, der(3) and der(22)t(4;22), the identity of which could only be clarified using FISH and/or microarray CGH. A full description of all results will be presented.

DISCUSSION: So far, our MLPA results are fully consistent with data from previous cytogenetic, FISH and/or microarray CGH analyses. We therefore anticipate that MLPA, as an adjuvant technique, may significantly contribute to routine prenatal cytogenetics, parallel to FISH a decade ago. The eventual evaluation of our MLPA approach with respect to its "functional" and "financial" consequences, in comparison to other conventional and molecular cytogenetic methods, will be discussed.

## 89. MATERNAL UNIPARENTAL DISOMY OF CHROMOSOME 16 IN A CASE OF SPONTANEOUS ABORTION

**K. Suzumori, Y. Kondo, S. Sonta, M. Tanemura, M. Sugiura**

1. Division of Human Reproduction and Development, Reproduction and Genetic Medicine, Nagoya City University Graduate School of Medical Science, JAPAN

OBJECTIVE: To investigate the involvement of uniparental disomies (UPDs) in spontaneous abortion.

DESIGN AND METHODS: We analyzed in detail the polymorphism of microsatellites on each chromosome in cases of abortion. Of the 52 spontaneous abortions investigated, 25 had a normal karyotype. The polymorphic analysis of these cases revealed that, in the villi from 24 of the 25 cases, biparental patterns were present in informative microsatellites in all autosomes. In the remaining case with a 46,XX karyotype, however, the informative patterns of the microsatellites of chromosome 16 appeared to be both of maternal origin. The results also showed that the region from the distal end of the short arm to near the middle point of the long arm of chromosome 16 (pter to D16S3107) were heterozygous, and those of the remaining region of the long arm (D16S3018 to pter) were homozygous.

RESULTS: That is, this fetus had maternal meiosis 1 nondisjunction of dyad 16 that accompanied a cross-over at near the middle point of the long arm. The present finding suggests that some UPDs may become a cause of spontaneous abortion.

## 90. FETAL CYTOGENETIC ANALYSIS OF FETUSES CONCEIVED BY INTRACYTOPLASMATIC SPERM INJECTION

**Zs. Szigeti, Z. Bán, Cs. Papp, E. Tóth-Pál, A. Beke, G. J. Joó, Z. Papp**

I. Department of Obstetrics and Gynecology, Semmelweis University, Budapest, HUNGARY

OBJECTIVE: The objective of this study was to determine the incidence of chromosomal anomalies in a cohort of ICSI pregnancies.

METHODS: Between 1996 and 2000 we examined 146 singular, 22 twin and 1 triplet pregnancies conceived by Intracytoplasmic Sperm Injection (ICSI). The patients were given prenatal genetic counseling and underwent genetic amniocentesis or chorionic villus sampling (CVS) and cytogenetic analysis. Amniocentesis was performed between 17<sup>th</sup> and 20<sup>th</sup> weeks of their pregnancy, CVS at the 11<sup>th</sup> week of pregnancy. We analysed the frequency of chromosome abnormalities and their relationship to parental age. The average maternal age was 34,6±3,7 years.

RESULTS: Amniocentesis/CVS and karyotyping was performed to evaluate the 193 fetuses from 169 ICSI pregnancies. Intrauterine karyotyping showed 5 cases of chromosome disorders out of the 146 singular pregnancies (3.4%). Three cases of trisomy 21, one case of trisomy 18 and one case of X monosomy were found. Out of the 22 twin pregnancies we found one case of trisomy 21 (4,45%), while in the triplet pregnancy all three fetuses had normal karyotypes. In case of maternal age over 35 years, we found 3 aneuploid fetuses of 80 patients (3,7%). In case of mothers younger than 35, we found 3 aneuploid fetuses of 89 patients (3,4%). We did not detect abnormal karyotype from the parents of the affected fetuses.

CONCLUSIONS: The ratio of chromosomal abnormalities seems to be slightly increased in ICSI pregnancies. We found no fetal aneuploidy in case of paternal age over 45. The incidence of aneuploid fetuses was not significantly increased in cases of mothers aged over 35. Our observation supports the need for fetal chromosome analysis of fetuses conceived by ICSI.

## 91. THE USE OF AMNIOCENTESIS AND QF-PCR TECHNIQUES FOR RAPID KARYOTYPE DIAGNOSIS IN LATE SECOND TRIMESTER AND THIRD TRIMESTER TO REPLACE CORDOCENTESIS

**W.W.K. To, A.M.Y. Chan, K.M. Mok**

Department of Obstetrics and Gynaecology, United Christian Hospital, Kowloon, Hong Kong, CHINA

**BACKGROUND:** When rapid fetal karyotyping is required in late gestations to facilitate obstetric management, cordocentesis and lymphocyte culture is traditionally performed to give rapid results. The availability of rapid quantitative fluorescence (QF)-PCR techniques allows amniotic fluid specimens to be used. Amniocentesis is less technically demanding and the transport of an amniotic fluid specimen is more convenient when the laboratory is in a separate location from the clinic.

**OBJECTIVE:** To evaluate whether the use of amniocentesis coupled with rapid QF PCR techniques can replace cordocentesis in rapid karyotype diagnosis in pregnancies diagnosed with structural abnormalities in late second and third trimester

**DESIGN AND METHODS:** Retrospective analysis of all cases with ultrasound diagnosed structural abnormalities in late second and third trimester over an 18-month period in a regional general obstetric unit. All amniotic fluid specimens were sent to a central laboratory about 18 km from the hospital. Specimens were tested with markers for chromosome 21, 13, 18 or X where appropriate.

**RESULTS:** There were a total of 5630 deliveries over the study period. Morphology scans were performed routinely between 18-20 weeks and significant structural abnormalities detected before 20 weeks were excluded from this analysis. Amniocentesis and QF-PCR was performed in 16 cases with significant structural abnormalities diagnosed between 21 and 30 weeks, including cardiac lesions, fetal hydrops, cystic adenoid malformation of the lungs, neck cyst, hydrocephalus, skeletal dysplasia and multiple abnormalities. All PCR reports were available within 24 hours. 10 were normal, but 3 cases showed trisomy 21 and 3 cases showed trisomy 18. Comparing subsequent foil culture results with the QF-PCR results showed no discrepancies in all cases, and no additional findings.

**CONCLUSIONS:** Late gestation amniocentesis together with QF-PCR could reliably replace cordocentesis to achieve rapid karyotype diagnosis.

## 92. FIRST TRIMESTER SCREENING OF FETAL STRUCTURAL ANOMALIES IN A GENERAL OBSTETRIC POPULATION

**G. Tsukerman<sup>1,2</sup>, O. Pribushenya<sup>1</sup>, G. Krapiva<sup>1</sup>, L. Lishtvan<sup>1</sup>, N. Venchikova<sup>1</sup>, S. Shreder<sup>1</sup>, S. Kovalev<sup>1</sup>, I. Solovyeva<sup>1</sup>, L. Savenko<sup>1</sup>, I. Novikova<sup>1</sup>, I. Kirillova<sup>1,2</sup>**

<sup>1</sup>Institute for Hereditary Diseases, Minsk, BELARUS

<sup>2</sup>Reproductive Genetics Institute, Chicago, USA

**OBJECTIVE:** The aim of this prospective study was to assess the effectiveness of first trimester screening by ultrasonography in detecting structural anomalies of the fetus in the general obstetric population.

**DESIGN AND METHODS:** During 1996–2003, 94,756 unselected pregnant women were screened for fetal malformation in the first trimester. The fetal anatomy was evaluated by transabdominal scan, and a transvaginal scan was performed when the visualization of fetal structures was suboptimal. 1.34% of pregnant women were under 18, and 6.1% were aged 35 or older. 90% of patients were screened at CRL within 38-67 mm, and 10% - at CRL of the fetus from 68 to 78 mm. The majority of the first-trimester patients underwent a routine second trimester ultrasound evaluation. Pathological examination was performed in 99% of cases that had an elective termination of pregnancy.

**RESULTS:** 2,174 empty sacs and non-viable fetuses and 959 multiple pregnancies were detected. Fetal anomalies were discovered in 346 cases, resulting in an overall incidence of 1 in 274 pregnancies. Together with routine findings (NTD), the ability to detect a range of rare monogenic malformations in the first trimester was demonstrated. During the study period, 900 malformed fetuses were aborted in the screened population, and 371 of them were identified in the first trimester. The first trimester detection rate for fetal malformations increased from 21% to 47% during the study period.

**CONCLUSIONS:** Nowadays, at least 50% of fetal structural anomalies can be detected in the first trimester in a general obstetric population.

### **93. AGE SPECIFIC RISK OF FETAL LOSS OBSERVED IN A SECOND TRIMESTER SERUM SCREENING POPULATION**

***P.R. Wyatt<sup>1</sup>, T. Owolabi<sup>2</sup>, A.M. Summers<sup>1</sup>, C. Meier<sup>1</sup>, T. Huang<sup>1</sup>***

<sup>1</sup>Genetics Program, North York General Hospital, Toronto, Ontario, CANADA

<sup>2</sup> Maternal Newborn Program, North York General Hospital, Toronto, Ontario, CANADA

OBJECTIVES: To investigate the age specific spontaneous fetal loss rates from mid-second trimester onward of pregnancies without known chromosomal or structural abnormalities.

DESIGN AND METHODS: The study involved 264,653 women screened between October 1995 and September 2000 with available pregnancy outcomes. Pregnancies associated with fetal chromosomal or structural abnormalities, insulin dependent diabetes mellitus, and multiple pregnancies were excluded. Women were grouped according to maternal age at expected date of delivery. Spontaneous fetal loss rates in each group were evaluated after adjusting fetal losses associated with amniocentesis, and identifiable ethnic groups.

RESULTS: Fetal loss rates were increased in both young and older women. The lowest rate was seen in women at mid-twenties. Compared with Caucasian and Asian women, Black women had higher fetal loss rate at nearly every age group.

CONCLUSIONS: The results of this study provided a baseline age specific spontaneous fetal loss rate of pregnancies within a specified gestational window.

### **94. FRAMING DECISIONS IN MULTIPLE PREGNANCIES AND OTHER COMPLEX SITUATIONS**

***M. Evans***

Institute for Genetics & Fetal Medicine, St. Luke's Roosevelt Hospital Center New York, USA

New technologies have allowed tens of thousands of couples to have access to methods to diagnose and treat selected abnormalities in utero. Counseling about such topics has become more complex as the number of options, frequently changing statistics, and variable availability of services has mushroomed.

How technologies become available to patients is a function of where and by whom they are developed, their complexity and costs, and patients' abilities to go to centers where they are available. How patients "hear" and internalize data varies as a function of their experiences, religious backgrounds, and value systems. Patients' willingness to undergo "experimental" treatments likewise varies by their commitment to the particular pregnancy, their perception of the relative utilities of various outcomes (e.g. no baby versus impaired baby), and their own psychological make up relative to "letting nature take its course" or aggressively challenging events.

## 95. HEMODYNAMICS AND HEART FUNCTION IN THE CHICK EMBRYO DURING DEVELOPMENT OF CARDIOVASCULAR MALFORMATIONS

*S. Stekelenburg-de Vos<sup>1</sup>, N.T.C. Ursem<sup>1</sup>, J.W. Wladimiroff<sup>1</sup>, B.C.W. Groenendijk<sup>2</sup>, R.E. Poelmann<sup>2</sup>*

<sup>1</sup>Department of Obstetrics and Gynaecology, Erasmus MC, Rotterdam, THE NETHERLANDS

<sup>2</sup>Department of Anatomy and Embryology, Leiden University Medical Center, Leiden, THE NETHERLANDS

**OBJECTIVE:** In the Netherlands approximately 1500 children are born with cardiac malformations each year. Therefore, we wish to obtain more insight into the etiology of cardiac malformations, especially into the relationship between hemodynamics and morphogenesis during cardiovascular development.

**DESIGN AND METHODS:** The chick embryo has been used as a model to study cardiac development because the embryonic chick heart resembles the developing human heart in many aspects. An intervention model for the chick embryo was designed in which specific cardiac malformations were induced by obstructing the right lateral vitelline vein with a microclip. In this venous clip model, Indian ink injections demonstrated altered intracardiac blood flow patterns, which lead to the observed malformations. Functional effects of clipping the right lateral vitelline vein on hemodynamics in the chick embryo were studied by measuring dorsal aortic blood flow velocities with a 20 MHz pulsed Doppler meter.

**RESULTS:** The main results demonstrate a significant decrease in hemodynamic parameters after venous clipping (study period 0-5 hours). Currently, heart function is being studied in the above model from ventricular pressure-volume loops, which are obtained by using a servo-null pressure system and video microscopy for blood volume estimation.

**CONCLUSIONS:** Above hemodynamic changes are also related to alterations in expression of shear stress responsive genes in pathways of important signaling molecules, such as endothelial NO-synthetase and endothelin-1.

## 96. RHESUS AND OTHER ISOIMMUNIZATIONS: AN UPDATE

*J. Queenan*

Department of Obstetrics and Gynecology, Georgetown University School of Medicine, Washington, DC, USA

At one time Rhesus isoimmunization was fatal for 50% of immunized pregnancies. Through a series of impressive diagnostic, therapeutic and preventative advances, today the likelihood of losing a fetus due to Rh-erythroblastosis fetalis is negligible. These extraordinary advances include AF DNA Rh-typing, AF deltaOD450, MCA peak systolic velocity, cordocentesis, fetal transfusions and, of course, prevention of immunization by Rh immunoglobulin (RhIG). In evaluating Rh-immunized pregnancies, the antibody level determines the degree of risk. MCA peak systolic velocity provides noninvasive fetal testing, but has limitations. There is still a role for AF deltaOD450 nm with AF Rh DNA typing. If Rh typing reveals that the fetus is Rh-negative, no further testing is necessary. A logical approach to fetal evaluation minimizes the amount of testing and provides effective fetal evaluation. Fetal transfusion is effective in treating fetal anemia and can reverse hydrops fetalis. Before the advent of Rh-immune prophylaxis, 14% of Rh-negative mothers delivering Rh-positive babies became immunized. Antepartum and postpartum RhIG markedly decreased the risk of immunization from 14% to 0.16%. There are still Rh-prophylaxis failures due to omission of RhIG or giving an inadequate dose.

Rh immune prophylaxis practices vary considerably worldwide. In many countries, the supply of RhIG is limited, and it may not be available when needed. There is underreporting of deaths due to Rh-immunization. Even in developed countries, cost may be a major factor in determining whether RhIG is available. Today, RhIG is made from serum of immunized human volunteers. In the future, development of a murine monoclonal RhIG could solve the shortage problem.

## 97. STEM CELLS FOR FETAL DIAGNOSIS AND THERAPY

*N. Fisk* (London, UK): Abstract not received

## 98. IN-UTERO HEPATOCYTE XENOTRANSPLANTATION FROM PIG TO LAMB

**P. Argibay, A. Lorenti, M. Barbich, D. Elias, S.H. Hyon, P. Farias**

Hospital Italiano de Buenos Aires, ARGENTINA

**OBJECTIVE:** Prenatal cell transplantation or cell xenotransplantation poses potentially significant advantages over postnatal therapies to treat inborn errors of metabolism. The fetal immune system is immature, incorporation into tissue-specific "niches" could be facilitated and early postnatal complications could be avoided. Based on the above mentioned considerations, our objective was to develop an in utero xenotransplantation model in which adult porcine hepatocytes were transplanted into fetal lambs.

**METHODS:** Single normal fetuses at 10 weeks of gestation were observed by ultrasound done on pregnant ewes. Under ultrasound control, the fetal abdominal cavity was accessed percutaneously and pig hepatocytes ( $40 \times 10^6$  of cells, 90% viability) injected. Fetal and mother haemodynamic parameters remained normal throughout the procedure. After recovery from anaesthesia, the ewes were returned to the animal facility until completion of pregnancy.

**RESULTS:** Ultrasound controls along the gestational period showed no alterations of the vitality or the morphology of the fetuses. Postnatal activity of transplanted hepatocytes was assessed by ELISA using a specific anti-porcine-albumin antibody. Serum pig albumin (mg/ml) of newborn transplanted lambs 1 day after birth were significantly higher than control lambs ( $p < 0,0001$ , "t" test). Protein electrophoresis of sera from control and transplanted lambs were compared with sera from donor pigs. Similarities in the migration patterns were observed only between the xenotransplant recipients and donor pigs. These results are very encouraging and at the present we have an active research program focused on in utero cell transplantation and xenotransplantation of hepatocytes and islets cells.

## 99. CYSTIC PERIVENTRICULAR LEUCOENCEPHALOMALACIA IN THE PRETERM INFANT

**B. Perti<sup>1</sup>, C. Fast<sup>2</sup>, M. Eder<sup>1</sup>, B. Resch<sup>2</sup>, B. Urlesberger<sup>2</sup>, J. Haas<sup>1</sup>**

<sup>1</sup>Department of Obstetrics and Gynecology and <sup>2</sup>Department of Pediatrics, Medical University Graz, AUSTRIA

**OBJECTIVE:** Periventricular leucoencephalomalacia (PVL) contributes significantly to neonatal mortality and morbidity affecting approximately 4-15% of preterm infants. The outcome of PVL is invariably unfavorable with motor as well as cognitive impairment. Between 60% and 100% of premature infants who have ultrasonographic findings considered to be characteristics of PVL later have cerebral palsy. Although the frequency of cerebral palsy has not declined, attention to some factors that are associated with an increased risk for PVL and cerebral palsy might help to prevent its development.

Our objective was to identify antenatal and intrapartum risk factors for PVL in premature infants and to investigate the relation between duration of membrane rupture and PVL. A summary of published studies about potential causes and risk factors for PVL will be given and potentially preventive strategies will be discussed.

**DESIGN AND METHODS:** During a time period from 1989 to 2001, 95 preterm infants with a gestational age from 26/0 to 35/0 developed PVL. 248 controls matched for gestational age and year of birth were selected.

**RESULTS:** The most important factor associated with an increased risk for PVL was prolonged rupture of membranes (OR 3.0 (95% CI, 1.8 – 4.9)). Preeclampsia (OR 0.3 (95% CI, 0.1 – 0.9)) and cesarean section without labor (OR 0.5 (95% CI, 0.3 – 0.8)) were associated with a reduced risk for PVL. There was no increased risk of cerebral palsy with maternal infection and histological chorioamnionitis. Infants born after more than 48 h from membrane rupture had a fourfold increase in the risk of PVL.

**CONCLUSIONS:** Infants born after prolonged rupture of membranes are at higher risk for developing PVL. The results of our study showed a significant correlation of the duration of membrane rupture and the occurrence of PVL. This finding might be useful for the obstetric management of preterm PROM after 28 weeks.

## 100. TREATMENT OF ACARDIAC TWINNING

### **W. Sepulveda**

Fetal Medicine Center, Clinica Las Condes, Santiago, CHILE

Management of a monochorionic twin pregnancy complicated by an acardiac twin is a major perinatal challenge. In a significant number of such pregnancies, the continuous growth of the acardiac twin and the associated "vascular steal" phenomenon lead to cardiac insufficiency and polyhydramnios in the pump twin. In such cases, intrauterine treatment to interrupt the blood supply to the acardiac twin may be the only way to prevent perinatal death of the pump twin.

Ablation of acardiac twins is a difficult task. Initial work focused on techniques targeting the umbilical cord, but technical difficulties to access the cord together with the high rate of death of the structurally normal pump twin have made this option less appealing to the perinatologists. Targeting the intrafetal vessels rather than the umbilical cord is another option. Colour-Doppler ultrasound allows a clear identification of the feeding single umbilical artery and the intraabdominal vessels in the acardiac twin, making these vessels readily accessible with ultrasound-guided needle techniques. Current techniques for intrafetal ablation include chemosclerosis with absolute alcohol, monopolar diathermy, interstitial laser, and radiofrequency. The most significant advantage of intrafetal ablation over funicular techniques is the fact that it can be performed in any fetal medicine unit with facilities to perform fetal blood sampling using needles commonly used for standard cytogenetic diagnostic procedures. In addition, a recent systematic review of the literature on acardiac twins treated with minimally invasive techniques (Tan & Sepulveda, *Ultrasound Obstet Gynecol* 2003;22:409-419) has shown that intrafetal ablation appears simpler, safer and more effective than cord occlusion techniques.

## 101. DEVELOPMENTS IN FETAL GENE THERAPY

### **C. Rodeck**

University College London, UK

Application of gene therapy *in utero* has been considered as a strategy for treatment or even prevention of early onset genetic disorders such as cystic fibrosis and Duchenne muscular dystrophy. Prenatal gene transfer may target rapidly expanding stem cell populations that are inaccessible after birth, permit induction of immune tolerance against vector and transgene and allow permanent gene transfer by use of integrating vector systems. Application of this therapy in the fetus must be safe, reliable and cost-effective. Recent developments in the understanding of genetic disease, vector design, and minimally invasive delivery techniques have brought fetal gene therapy closer to clinical practice. Prenatal studies in animal models are being pursued in parallel with adult studies of gene therapy, but they remain presently at the experimental stage.

## 102. FALSE-POSITIVES IN THE PRENATAL ULTRASOUND SCREENING FOR FETAL MALFORMATIONS

### **T. Cobo, M.A. Martínez-Zamora, A. Borrell, B. Puerto, J.A. Martínez-Crespo, V. Borobio, F. Botet, A. Nadal, A. Albert, V. Cararach, J.A. Vanrell**

Unitat de Diagnòstic Prenatal, Institut Clínic de Ginecologia, Obstetrícia i Neonatologia, Hospital Clínic de Barcelona, SPAIN

**OBJECTIVE:** To evaluate the false-positive cases of fetal malformations in the ultrasound pregnancy screening.

**DESIGN AND METHODS:** In our center, we have recorded prospectively all the diagnoses of fetal malformations suspected prenatally by ultrasound examination from January 1994 to December 2003. It also includes the defects found at birth which have not been previously identified and the false-positive cases recorded before birth but not confirmed by examination postnatally.

**RESULTS:** A total of 1166 cases of fetal malformation have been registered. There has been 72 cases of malformations suspected initially but not confirmed at birth. The false positives were distributed as follows: 19 cases of nephrourologic malformations (26.4%), 15 cases of skeleton malformations (20.8%), 13 cases of digestive malformations (18%), 11 cases of neurologic malformations (15.2%), 5 cases of genital malformations (6.9%), 5 cases of cardiac malformation (6.9%) and 4 cases of respiratory malformations (5.5%).

**CONCLUSIONS:** The rate of false-positive in our serie was 6.17%. The systems mainly involved were the nephrourologic, skeletal, digestive and central nervous system.

### 103. HOW PAINFUL IS AMNIOCENTESIS?

**Á. Csaba, Z. Bán, J.G. Joó, L. Lázár, Z. Papp**

I. Department of Obstetrics and Gynecology, Semmelweis University, Budapest, HUNGARY

OBJECTIVES: To measure the level of pain and anxiety associated with amniocentesis (AC).

METHODS: We prospectively administered a questionnaire about pain and anxiety to 184 women undergoing AC at the I. Dept. of Obstetrics and Gynecology of Semmelweis University. The level of pain was quantified with numerical and pictorial scales and the degree of anxiety with a numerical scale (0-100 in increments of 10).

RESULTS: The mean pain score for AC,  $29.2 \pm 20.5$  was low. A higher degree of anxiety was associated with younger maternal age and nulliparity. A higher degree of anxiety was associated with a higher level of pain and those who had less previous pregnancies. The level of pain appeared to be higher among patients who were more obese.

CONCLUSION: In general, amniocentesis is associated with a tolerable amount of pain. In certain groups of patients the procedures may be associated with higher levels of pain and/or anxiety.

### 104. LONGITUDINAL VOLUME MEASUREMENTS OF THE HUMAN SECONDARY YOLK SAC USING THREE DIMENSIONAL ULTRASOUND: PRELIMINARY RESULTS

**K.A.J. de Clippel, M.J.N.C. Wijman, P.C. Struijk, J.W. Wladimiroff, E.A.P. Steegers**

Obstetrics and Gynecology, Erasmus University Medical Center, Rotterdam, THE NETHERLANDS

OBJECTIVE: To obtain longitudinally volume measurements of the human secondary yolk sac in relationship to gestational age.

DESIGN AND METHODS: Five healthy women with singleton pregnancies were recruited. During the first trimester, transvaginal ultrasound scans (5-7 MHz probe) were performed weekly from the 6th until the 13th week of gestation using a Voluson 730 Expert (GE Medical Systems, Kretztechnik, GmbH 7 OHG, Austria). All data were recorded on CD and analysed off line using VOCAL (Virtual Organ Computer-aided Analysis). Written informed consent was obtained from every subject.

RESULTS: At 6 weeks of gestation the mean (SD) yolk sac volume was 45.3 (18.5) microliter. The maximum volume was reached at 10 weeks: 130.5 (34.6) microliter, followed by a subsequent decrease in the next weeks. By 13 weeks of pregnancy no yolk sac was detectable any more in all cases.

CONCLUSIONS: With three dimensional ultrasound yolk sac volume can be determined accurately. It is hypothesized that these measurements can be used in the near future to discriminate between normal and pathological development of early pregnancy.

## **105. UTERINE ARTERY DOPPLER VELOCIMETRY IN LOW-RISK NULLIPAROUS WOMEN AND IN PREGNANCIES COMPLICATED BY DIABETES MELLITUS ( TYPE 1 AND 2 )**

***J. Dienes, G. Nagy, K. Kékes***

Borsod-Abaúj-Zemplén County Hospital, Miskolc, HUNGARY

**OBJECTIVE:** The examination of the utero-placental circulation is a useful method to decrease the perinatal morbidity and mortality. It is especially advisable in high-risk pregnancies. Patients suffering from diabetes mellitus create an important part of pathologic pregnancies. Among them the frequency of spontaneous abortion, fetal demise, gestational hypertension, preeclampsia are higher.

**DESIGN AND METHODS :** Authors examined the uterine artery Doppler velocimetry in 20 diabetic patients (16 patients type 1 and 4 patients type 2) at the 11-12 weeks of gestation. The examination was repeated at the 18-20 weeks of gestation and later at the 24, 28 and finally at the 34 weeks of gestation (group I). The results were analyzed and compared with the data of 34 low-risk pregnant women (group II). All of the patients were nulliparous with healthy pregnancies. Patients with fetal malformations were excluded from the study.

**RESULTS:** The uterine artery Doppler velocimetry at the 11-12 weeks of gestation didn't show any differences between the two groups. Repeating the examination 7 weeks later there was a slight difference. Patients suffering from diabetes type 1 produced higher resistance indices than low-risk patients but the difference was not significant.

At the 24 weeks of gestation 3 diabetic patients type 1 developed bilateral notches but only 1 in the low-risk group. All the four patients developed preeclampsia subsequently. Unilateral notches were diagnosed in five patients (3 pregnant woman with diabetes mellitus type 1 and 2 low-risk patients). Neither hypertension nor preeclampsia were diagnosed in their cases. On the whole 3 patients with preeclampsia and 3 patients with gestational hypertension were diagnosed in the group I. In the group II 1 preeclampsia and 2 gestational hypertension were diagnosed.

**CONCLUSIONS:** The uterine artery Doppler velocimetry is a useful method in the screening of preeclampsia. The velocity waveforms in diabetic patients don't differ from the ones in healthy patients. As gestational hypertension and preeclampsia are more frequent in diabetic patients the Doppler examination of the uterine arteries is essential during pregnancy.

## 106. PERINATAL OUTCOMES REGARDING TO THE RISK FACTORS AND THE EFFICACY OF THE TREATMENT OF DIABETES MELLITUS

**Z. Garamvölgyi, I. Krasznai, J. Hidvégi, J. Rigó Jr.**

I. Department of Obstetrics and Gynecology, Semmelweis University, Budapest, HUNGARY

**OBJECTIVE:** To examine the associations between the maternal carbohydrate metabolic disorders (pregestational and gestational diabetes also) and their perinatal outcomes regarding to the risk factors and the efficacy of the treatment of diabetes.

**DESIGN AND METHODS:** From december 2002 to march 2003, 242 pregestational and gestational diabetic pregnant women were examined. Of the 242 pregnancies entered in our retrospective study the first trimester 18 treated by diabetes type 1 and 6 by diabetes type 2. In 217 pregnancies associated with gestational diabetes mellitus (GDM) insulin or only diet were used for treatment. An oral glucose tolerance test (OGTT) using WHO's recommendation (75g) was also administered for the recognition of GDM. Pregravid body weight was obtained and BMI ( $\text{kg}/\text{m}^2$ ) was used measure overweight and obesity. The maternal age, the parity, the risk factors of diabetes and the mode of delivery were matched to the effect of diabetes treatment. All participating patient's neonates' outcome were examined: birth weight and neonatal hypoglycaemia, jaundice, polycythaemia and hypocalcaemia complicated maternal diabetes. All the cases of malformation were examined. **RESULTS:** The highest pregravid weight and average BMI value accompanied the group type 2 diabetes ( $28.41 \text{ kg}/\text{m}^2$ ) following by the group of GDM ( $27.33 \text{ kg}/\text{m}^2$ ). Pregnancies associated gestational diabetes mellitus treated dietary in 38% (82/217) and obtained insulin therapy in 62% (135/217) of cases. The induction of insulin therapy in half of the GDM patients was between the 25 and 32 gestational week (50.7%). In 73.3% of (99/135) cases of insulin dependent GDM insulin therapy needed once daily only opposite to the 36 cases (36/135) where insulin supplement obtained shared a day. Previous GDM in the patient's history was a risk factor to the onset of a gestational diabetes treated by insulin (14/135). The frequency of caesarean delivery was increased both the pregestational and gestational diabetes group of participates. **CONCLUSIONS:** Insulin dependent GDM could predict the later onset of type 2 diabetes. Because of the high prevalence of GDM in obese women, maintenance of normal body weight is recommended. Insulin is the pharmacologic therapy that has most consistently been shown to reduce fetal morbidities.

## 107. IN UTERO PANCREATIC ISLET XENOTRANSPLANTATION DEVELOPS AN AUTOIMMUNE DIABETES LIKE PROCESS

**M. Garcia, P. Argibay, A. Hidalgo, M. Barbich, M. Vieiro, S.H. Hyon, P. Farias**

Hospital Italiano de Buenos Aires, ARGENTINA

**INTRODUCTION:** In utero cell transplantation may serve as a tool of research regarding the physiopathology of autoimmune diseases, such as diabetes.

**OBJECTIVE:** To evaluate the engraftment and the immune reaction induced by human islets transplanted in utero to ovine fetuses.

**DESIGN AND METHODS:** Human islet cells were transplanted in utero to fetal lambs at 17 weeks of gestational age. Insulin, glucose and intravenous glucose tolerance tests (IVGTT) were determined in the pregnant ewes after the transplant procedure and both in the ewe and the lamb after its birth. Anti-islet (ICA) and anti-insulin (IAA) antibodies were investigated. Inflammatory or degenerative phenomena were assessed in pancreatic biopsies from the mother and the lamb. Non-transplanted animals were used as controls.

**RESULTS:** Significant hyperglycemia (transplanted ewes vs. controls,  $150$  vs.  $50 \pm 2$  mg/dl, respectively), abnormal 1VGTT, and high titers of antibodies (IAA +, and ICA  $> 512$  JDF) were observed in the ewes, which tended to normalize upon giving birth, although hyperglycemia persisted. No histologic alterations were found in the native pancreas. Regarding the recipient lambs, significant hyperglycemia, abnormal IVGTT, and antibodies (IAA+, ICA  $> 1028$  JDF) were seen after birth. The postnatal biopsy of the native pancreas showed an inflammatory process. In the subsequent months, hyperglycemia and antibodies persisted with a progressive increase of circulating insulin (subject vs. controls, basal/stimulated,  $46,9/201$  vs.  $20,5/84$  microU/ml, respectively), with a progressive decrease in the islet number of the native pancreas.

**CONCLUSIONS:** Our results suggest that in utero xenotransplantation of human islets to a fetus induces an immune cross-reaction that affects both the mother and the recipient. The original model presented here could be interesting to study experimental diabetes and related fetus-maternal immunological reactions.

## **108. LONGITUDINAL DETERMINATION OF PLACENTAL VASCULARIZATION INDEX DURING THE SECOND HALF OF PREGNANCY, USING 3-D POWER DOPPLER ULTRASOUND; FIRST RESULTS OF A PILOT STUDY**

***M. Groenewout, M.J.N.C. Wijman, P.C. Struijk, J.W. Wladimiroff, E.A.P. Steegers***

Obstetrics and Gynecology, Erasmus University Medical Center, Rotterdam, THE NETHERLANDS

OBJECTIVE: To study longitudinally changes in placental vascularization index in uncomplicated pregnancies in comparison with pregnancies complicated by preeclampsia. Using 3-D ultrasound in combination with power Doppler, numeric information on the percentage of moving bloodcells within the total placental volume can be obtained.

DESIGN AND METHODS: Three patients with uncomplicated singleton pregnancies were recruited from the ErasmusMC out-patient clinic. Ultrasound examination was performed at 24, 28 and 32 weeks of gestation. Three hospitalised patients with mild preeclampsia were studied once. The placental vascularization index was determined on the number of color voxels relatively to the total number of voxels, using the 3-D Volusion 730 Expert (GE medical systems, Kretztechnik) ultrasound machine with prefixed factory settings.

RESULTS: During uncomplicated pregnancy, the VI increased from 9.3 % (SD = 2.1%) at 24 weeks to 12.6% (SD = 4.6%) at 28 weeks, followed by a decrease to 7.8% (SD = 3.7%) at 32 weeks. Corrected for gestational age, preeclamptic patients showed a VI which was 0.8 SD below the mean of the control group (Z-scores 0.79 (26 wks), 0.96 (29 wks) and 0.73 (33 wks) respectively).

CONCLUSIONS: After an initial increase, VI seems to decrease during the early third trimester. Pregnancies complicated by preeclampsia display a relatively low vascularisation index.

## **109. CONGENITAL HEART DISEASES IN TWIN PREGNANCIES**

***J. Hajdú, A. Beke, T. Marton, E. Hruby, B. Pete, Z. Papp***

I. Department of Obstetrics and Gynecology Semmelweis University Budapest, HUNGARY

OBJECTIVE: To found connection between the type of congenital heart malformations and twin pregnancies.

DESIGN AND METHODS: Retrospective analysis of data of fetal cardiology database between 1. January 1996 and 30. November 2003.

RESULTS: In 22534 singular pregnancies 455 (2,02%), and in 653 twin pregnancies 31 (4,6%) severe congenital heart malformations were diagnosed prenatally. In monozygotic twin pregnancies 36% of heart malformations were pulmonary stenosis and 45% endocardial fibroelastosis. In dizygotic twin pregnancies Ebstein malformation was more common than it statistically expected. In dichorionic and dizygotic twin pregnancies the cardiac malformations were similar to in singular pregnancies.

CONCLUSIONS: In twin pregnancies the rate of congenital heart malformations was higher than in singular pregnancies, that's why the twin pregnancy is indication for fetal echocardiography. In monochorial twin pregnancies type of congenital heart malformations was different from those found in singular, dizygotic or dichorionic twin pregnancies. The chorionicity seems more important than the zigozity.

## **110. CONGENITAL DIAPHRAGMATIC HERNIA: CHANGING THE PATIENTS' ADMISSION TO GENETIC COUNSELLING ON THE BASIS OF 24 YEARS DATA**

**Á. Harmath, Z. Papp**

I. Department of Obstetrics and Gynecology, Semmelweis University, HUNGARY

OBJECTIVE: To retrospectively analyse the files of our patients with congenital diaphragmatic hernia on the basis of our database.

DESIGN AND METHODS: We analysed the data of 179 patients who attended genetical counselling between 1979-1990 in the East Hungarian region and 1991-2002 at our Department. The patients' number was 35.5% higher in the second period. Patients were referred to outpatient clinic either because of fetus or newborn with congenital diaphragmatic hernia in the history or the suspicion of fetal malformation in the current pregnancy. The study focuses on changes of admission routine, mother's age and gestational age at diagnosis, as well as on recent data of diagnosed cases.

RESULTS: In the first period 76 while in the second 103 patients attended the counselling. The mother's age at the first admission was 26.7 and 26.3 years respectively. In the first period 80.3% of the cases were transferred to counseling with the history of malformation. In the second twelve years period the main cause in 41.7% of the patients was the ultrasound signs of malformation. There are no data referring to the cause of admission in 23.3% of all cases in the last period. Taking this into account the difference is 54.4%. The average gestational age at the time of diagnosis was 26.38 weeks in the first and 25.15 weeks in the second period. Parasternal diaphragmatic hernia was diagnosed in 7.7% in the first and 11.11% in the second period. Bilateral hernia was diagnosed in one case. Detailed data are available from the second twelve years; during this period the rate of associated malformations was 34.2%.

CONCLUSION: There was no significant difference in maternal age at first admission in spite of the fact that in the last years the age of first pregnancy has been postponed. The main reason of referral to genetic counselling has changed; nowadays the ultrasound diagnosis of congenital malformation has become significantly higher. With the technical development and with the improvement of searcher skills the average gestational age at the time of diagnosis has become one week earlier in the second period.

## **111. INTRAUTERINE ULTRASOUND GUIDED LASER FOTOCOAGULATION FOR ACARDIAC TWIN**

**K. Hodík<sup>1</sup>, I. Musilová<sup>1</sup>, J. Nateková<sup>1</sup>, P. Eliáš<sup>2</sup>, M. Podholová<sup>1</sup>**

<sup>1</sup>Department of Obstetrics and Gynecology, <sup>2</sup>Department of Radiology, University Hospital, Charles University, Faculty of Medicine in Hradec Kralove, CZECH REPUBLIC

OBJECTIVE: Twin-reversed arterial perfusion (TRAP) sequence is a rare anomaly that occurs in 1 of monochorionic twins and 1 in 35 000 pregnancies overall. This condition is characterized by a severely malformed fetus with an absent heart. Flow occurs from the normal „pump" twin through vascular anastomoses to the acardiac twin via a single placenta. It puts the „pump" fetus at risk of high-output cardiac failure with the perinatal mortality rate 50-70%. Alternatives of management include: no intervention, termination of pregnancy, medical management with maternal administration of digoxin or indomethacin and interruption of blood flow to the acardiac twin (hysterotomy and removal of the abnormal twin, umbilical cord ligation, embolization or fotocoagulation under ultrasonic or fetoscopic guidance).

DESIGN AND METHODS: We report a case of TRAP sequence diagnosed at 10+6 weeks of gestation with progressive growth of the acardiac twin. At 17+0 weeks of gestation, ultrasound guided laser fotocoagulation of the intraabdominal umbilical artery and the abdominal aorta of the acardiac twin was performed.

RESULTS: The procedure interrupted perfusion and stopped growth of the acardiac twin. Serial sonographic evaluation demonstrated normal development of the „pump" twin. A healthy female infant (3260g/49cm) was born vaginally at 38+3 weeks of gestation.

CONCLUSIONS: The ultrasound guided laser fotocoagulation seems to be a safe and an effective method of treatment for TRAP sequence. It should be preferred to pregnancy termination, fetoscopic guided methods and conservative management of cases with progressive growth of the acardiac twin.

## 112. ISOLATED FETAL ASCITES DETECTED BY SONOGRAPHY

*J. Horovitz, C. Deckindt, R. Mangione, F. Guyon, R. Saura*

Centre de Diagnostic Prénatal, Maternité, Hopital Pellegrin, Bordeaux, FRANCE

Chylous fetal ascites is a rare symptom easily diagnosed on ultrasound examination. It is due to primary lymphangiectasia, a generalized malformation of the lymph vessels. Management requires a thorough etiological work-up in order to eliminate all other possible causes of fetal ascites. Once diagnosis has been confirmed, symptoms are treated according to fetal and maternal tolerance, and clinical course followed-up by ultrasound scans.

We report the case of a 32-year-old, gravida 1 para 0 Caucasian woman. Her pregnancy was uneventful till 22 WG when isolated fetal ascites was diagnosed. There were no additional fetal anomalies and fetal measurements were within the normal range. Amniocentesis for fetal karyotyping had been performed at 20 WG because of positive serum markers. During etiological work-up a second amniocentesis was performed and revealed no anomaly. At 23 WG 55 ml of fluid were evacuated, and the diagnosis of chylous ascites was made. Chylous ascites reformed and led to three subsequent evacuations, 200 ml at 28.4 WG, 1.2 liters at 30.4 WG, and 400 ml at 35.5 WG just before cesarean section which was performed for poor maternal and fetal tolerance to ascites. A girl weighing 2700 grams was born and immediately underwent evacuation of 600 ml of ascites. Three months later the infant had undergone 7 ascites punctures and was on parenteral feeding. DISCUSSION: The diagnosis of ascites is easy to perform and an etiology must be searched for. According to the cause of ascites, termination or continuation of pregnancy are discussed. The difficulty lies in assessing the baby's prognosis.

CONCLUSION: Early prenatal diagnosis of fetal ascites is important because some cases can be cured in-utero. In the case of developing hydrops due to immunological cause, if treatment is instored early on prognosis is better. Other non-curable etiologies, or etiologies associated with poor prognosis, lead to consider termination of pregnancy.

## 113. TWIN PREGNANCIES COMPLICATED BY INTRAUTERINE DEATH OF ONE CO-TWIN: MATERNAL RISKS OF EXPECTATIVE MANAGEMENT

*E. Hruby*

I. Department of Obstetrics and Gynecology, Semmelweis University, Budapest, HUNGARY

OBJECTIVES: To examine the specific maternal risks and outcomes of expectative management in multiple pregnancies with single fetal death.

STUDY DESIGN: Case-controlled, retrospective study of 63 consecutive pregnancies (twins, triplets) with evidence of antenatal fetal death, managed expectatively from 1<sup>st</sup> July 1990 to 31<sup>st</sup> December 2001 in our institute compared with 126 twin pregnancies with liveborn siblings matched for age of pregnancy and parity. The intrauterine death were detected by ultrasonography. Observations included the occurrence of specific maternal complications during pregnancy, labour and delivery, and postnatal period, the gestational age and parity, and the sex of the twins and the chorionicity of the placenta.

RESULTS: The gestational age at delivery was 24-39 weeks, parity 1-6. Maternal coagulopathy and other specific complications were not observed during pregnancy, labour and delivery, and postnatal period. There were no significant difference between the two study groups

CONCLUSION: The lack of the specific maternal complications associated with twin pregnancies complicated by intrauterine death of one co-twin support the idea of the expectative management. The continuation of pregnancy prevents the extreme prematurity and improves the perinatal data without to increase maternal risks.

#### **114. PRENATAL DIAGNOSIS, PHENOTYPIC AND OBSTETRIC CHARACTERISTICS OF HOLOPROSENCEPHALY**

**J.G. Joó, A. Beke, Cs. Papp, E. Tóth-Pál, Zs. Szigeti, Z. Bán, Z. Papp**

I. Department of Obstetrics and Gynecology, Semmelweis University, Budapest, HUNGARY

The analysis of the 50 cases of holoprosencephaly we encountered between 1981 and 2000, including the anatomical, diagnostic and clinical aspects, as well as the associating craniofacial malformations, form the essence of our poster. The diagnosis of fetal malformations, especially those of the central nervous system is strikingly important in the practice of genetic counselling. Early diagnosis of holoprosencephaly is very significant, not only because of the prognosis, but also because of the emotional effects caused by the accompanying craniofacial malformations. The associated craniofacial malformations, like cyclopy, hypotelorism, proboscis or cleft palate have a great diagnostic and prognostic value. In 12% of the cases, holoprosencephaly occurred in an isolated form, while in the majority of the cases (88%) with an accompanying (mainly facial) malformation. The malformations of the central nervous system and other organs that accompany holoprosencephaly are also recapitulated. The summary of the obstetrical and diagnostical characteristics should be useful in the management of holoprosencephaly.

#### **115. HEREDITARY LONG QT SYNDROME IN PREGNANCY. ANTENATAL AND INTRAPARTUM MANAGEMENT OPTIONS**

**I. Katsoulis, I. Papageorgiou, N. Papantoniou, A. Antsaklis**

I. Department of Obstetrics and Gynaecology, University of Athens, "Alexandra" Regional General Hospital, Athens, GREECE

**OBJECTIVE:** We report a case of hereditary long QT syndrome in pregnancy and the management options used for the antenatal and intrapartum care of the patient. Our aim is to present this rare but severe form of arrhythmia, how this can complicate pregnancy, and most importantly to present safe options of management.

**CASE REPORT:** The patient is a 26 year-old primigravida suffering from the hereditary form of the long QT syndrome. She is carrying a pacemaker and is been treated with b-blockers. During the antenatal period she had a joint antenatal care given by an obstetrician and a cardiologist with regular monthly visits at the high risk pregnancy clinic of our hospital, and serial assessment of both her cardiac function and of the well being of the fetus with CTG, ultrasound and doppler studies. At 36 plus 6 days of gestation she finally had an elective lower segment caesarean section under general anaesthesia in order to avoid the strain related to a vaginal birth. Postnatally she had an uncomplicated recovery and the neonate underwent a thorough assessment with ECG and heart ultrasound studies that haven't shown any evidence of cardiac disease as yet. Genetic counseling was offered to the couple as well.

**DISCUSSION:** The long QT syndrome is a serious cardiac arrhythmia more often seen in women. This is a very serious cardiac arrhythmia that is characterized mainly by prolongation of the QT waveform on the ECG and clinically usually presents in childhood and early adolescence with recurrent episodes of loss of consciousness and even sudden cardiac death. There is a hereditary form that has been related with mutations of six genes and an acquired form caused by antiarrhythmic drugs. The patient that suffers from the hereditary form of the disease usually enters pregnancy already diagnosed and treated accordingly. Joint antenatal care between an obstetrician and a cardiologist is needed all throughout, that should include continuation of the therapy and serial assessment for the well being of the mother and fetus. Recently, fetal magnetocardiography has been proposed for antenatal diagnosis of the syndrome. The risk that the syndrome can cause cardiac decompensation of the mother is increased during labour and immediate postpartum period. An elective caesarean section under general anaesthesia is a safe option for delivery, and surveillance for the mother and the neonate should continue postnatally. The latter should have extensive cardiac assessment and genetic approach for one or more gene mutations that have been associated with the syndrome.

## 116. FETAL CONSEQUENCES OF OPIATE USE IN PREGNANCY

**Z. Kovács, J. Rigó Jr**

I. Department of Obstetrics and Gynecology, Semmelweis University, Budapest, HUNGARY

OBJECTIVE: To evaluate the perinatal outcomes following maternal opiate use in pregnancy.

DESIGN AND METHODS: A retrospective analysis of 12 pregnancies of 11 opiate-addicted women giving birth at the I. Department of Obstetrics and Gynecology, Semmelweis University, between 1993-2003.

RESULTS: Of the women, 25 % registered before the 20<sup>th</sup> week of pregnancy and 75 % during the 20<sup>th</sup>-33<sup>rd</sup> week of pregnancy. In the third trimester of pregnancy six women took methadone and four women took clonazepam, often together with heroin, cannabis, amphetamines or benzodiazepines. Only three women succeeded in quitting totally opiate-abuse. There were 12 pregnancies, which resulted in 12 live births (only 3 preterm deliveries). There were 3 cases of intrauterin growth retardation, only once associated with preterm delivery. The HCV-Ab tests were positive for three heroin-dependent women, at the same time VDRL, HPV identification and HAV-Ab test were positive only once. None of the patients were HIV and HbsAg positive. Postpartum six opiate-dependent women and their neonates needed to be treated with substitution therapy. None of the neonates was HCV-Ab, HAV-Ab, HBSAg-, VDRL, HIV-positive. We managed to prevent the stillbirth and the perinatal death.

CONCLUSIONS: The treatment of pregnant opioid-dependent women is of great importance to avoid the severe maternal and perinatal complications of opiate-abuse.

## 117. ANDROGENS AS MARKERS OF PREECLAMPSIA

**I. Krasznai, Gy. Szendei, Z. Garamvölgyi, N. Dévényi, T. Böze, J. Rigó Jr.**

I. Department of Obsterics and Gynecology, Semmelweis University, Budapest, HUNGARY

OBJECTIVE: There is a probable relation between serum level of testosterone and endothelial dysfunction.

DESIGN AND METHODS: Blood samples were collected from 20 preeclamptic and 20 normotensive pregnant in the III. trimester. The definition of preeclampsia was on the suggestion of ACOG (2002). There was no patient with chronic hypertension, renal disease, and diabetes mellitus in the two groups. The quantity determination of serum testosterone was with RIA (Radio Immuno Assay) and the SHBG with IRMA (Immuno Radiometric Assay). The hyperandrogen symptoms (acne, loss of hair, hirsutism) were collected by retrospective questions. The statistical calculations were made with two-sample t probe and with the use of  $\chi^2$  tests.

RESULTS: The serum level of testosterone was significant higher in patients with preeclampsia than in normotensive pregnant. There was no correlation of serum level of SHBG between the two groups. The hyperandrogen symptoms were in 9/20 vs. 3/20. There was no significant differentiation between the birth weight from preeclamptic and normotensive deliveries.

CONCLUSIONS: The higher level of serum testosterone can contribute to the endothel cell damage and the to the development of preeclampsia.

## **118. CLINICAL SIGNIFICANCE OF SUBCHORIONIC AND RETROPLACENTAL HAEMATOMAS DETECTED IN THE FIRST TRIMESTER OF PREGNANCY**

**S. Nagy, M. Bush, R. Lapinski, S. Gardó**

Petz Aladár County Hospital, Department of Obstetrics and Gynecology, Győr, HUNGARY

Mount Sinai Hospital, Department of Obstetrics and Gynecology, New York, NY, USA

**OBJECTIVE:** To evaluate the long-term clinical significance of intrauterine haematomas detected in the first trimester of pregnancy in a general obstetric population.

**DESIGN AND METHODS:** A prospective study was designed to compare perinatal outcomes in 187 pregnant women with intrauterine hematomas and 6488 controls in whom hematomas were not detected.

**RESULTS:** The incidence of intrauterine hematoma in the first trimester in a general obstetric population was 3.1%. A retroplacental position of the hematoma was significantly correlated with an increased risk for adverse maternal and neonatal complications. The presence or absence of symptoms of threatened abortion did not affect these outcomes. The rates of operative vaginal delivery (RR: 1.9 ; CI: 1.1, 3.2) and cesarean delivery (RR: 1.4 ; CI: 1.1, 1.8) as well as the rates of pregnancy-induced hypertension (RR: 2.1 ; CI: 1.5, 2.9) and preeclampsia (RR: 4.0 ; CI: 2.4, 6.7), were significantly greater in the hematoma group. Placental abruption (RR: 5.6 ; CI: 2.8, 11.1) and placental separation abnormalities (RR: 3.2 ; CI: 2.2, 4.7) were also significantly more frequent in the hematoma group. Perinatal complications, including the rate of preterm delivery (RR: 2.3 ; CI: 1.6, 3.2), fetal growth restriction (RR: 2.4 ; CI: 1.4, 4.1), fetal distress (RR: 2.2 ; CI: 1.7, 2.9) and neonatal intensive care unit admission (RR: 5.6 ; CI: 4.1, 7.6), were also significantly increased in this group. Furthermore, the frequency of intrauterine demise and perinatal mortality was increased in the hematoma group, but this difference did not reach statistical significance ( $P_s = 0.6$  and  $0.2$ ).

**CONCLUSIONS:** Our study suggests that the presence of an intrauterine haematoma during the first trimester may identify a population of patients at increased risk for adverse pregnancy outcome.

## 119. PRENATAL DIAGNOSIS OF JOINT CONTRACTURES: KARYOTYPE, ASSOCIATED FINDINGS AND OUTCOME

*E. Pajkrt, LS. Chitty*

Fetal Medicine Unit, University College London Hospital, London, UK

**OBJECTIVE:** Congenital joint contractures are fixed flexion or extension deformities of the knee, elbow, wrist, hand or ankle. Contractures can occur in one joint, or in multiple joints as a consequence of variable etiology. The aim of this study was to investigate the karyotype, associated findings and fetal outcome in prenatally diagnosed joint contractures.

**DESIGN AND METHODS:** A retrospective study was performed of all prenatally diagnosed cases with joint contractures in which postnatal follow-up was available between January 1992 and June 2003.

**RESULTS:** 284 cases were identified. There were 14 (5%) cases with isolated unilateral talipes. In this group no chromosomal or other anomalies were detected postnatally. 35 (12%) fetuses had isolated bilateral talipes and 13 (37%) of those had invasive testing, but no chromosomal anomalies were found. However 2 cases were diagnosed with multiple anomalies postnatally. Talipes, in combination with other structural anomalies or markers for aneuploidy was found in 53 (19%) cases. Karyotyping was performed in 38 (72%) and 11 (20%) were abnormal. An unfavourable fetal outcome was present in another 12 (21%). Although the majority of surviving cases showed isolated talipes postnatally, there were also several genetic syndromes diagnosed, such as Smith-Lemli-Opitz, Stickler and Pierre Robin. Spina bifida in combination with talipes was present in 47 (17%). One fetus had trisomy 18. Rocker bottom feet were found in 20 (7%) fetuses usually in combination with clenched hands. All of these fetuses had other anomalies. Chromosome analysis revealed an abnormal karyotype in 11 (55%) (mainly trisomy 18). There were no survivors in this group. The 3 cases that were not terminated because of an abnormal karyotype or multiple anomalies resulted in intra-uterine or neonatal death. Complex contractures of different combinations of joints were present in 115 (40%) cases. 95 (83%) had karyotyping and 26 (23%) showed an abnormal karyotype. The outcome of the majority of cases was poor, showing a large spectrum of genetic syndromes such as Pena-Shokeir, arthrogryposis, Holt-Oram, COFS and Cornelia de Lange. Also in a significant number of fetuses a final diagnosis could not be made.

**CONCLUSIONS:** The outcome of prenatally diagnosed isolated talipes is good, although a thorough search for other possible anomalies should always be performed. Talipes in combination with other structural anomalies or markers of aneuploidy significantly increases the risk of aneuploidy and adverse fetal outcome. Rocker bottom feet never presented as an isolated finding in our study and were always associated with a poor prognosis. Complex joint contractures are a manifestation of a very broad spectrum of conditions associated with aneuploidy, myopathy, neuropathy and genetic syndromes.

## 120. FIRST TRIMESTER ULTRASONOGRAPHY SCREENING FOR FETAL ABNORMALITIES: 24 YEARS OF STUDIES

### A. Papitashvili

Department of Obstetrics and Gynecology, Tbilisi State Medical University, Tbilisi, GEORGIA

**OBJECTIVE:** The aim of our prospective study was to evaluate the effectiveness of routine first level ultrasound examination useful in daily clinical practice of standard maternal hospitals without expert category ultrasound machines, to detect the fetal abnormalities in first trimester of pregnancy.

**DESIGN AND METHODS:** During the period of study from 1980 till 2004 the 75800 patients were examined in first trimester of pregnancy between 10 and 15 weeks excluding preliminary clinical/genetic counseling. The age of patients ranged from 14 to 45 years (mean=27.2). 20115 patients (26.5 %) had two gestations, 45340 patients (59.8 %) were of their first gestation, 10345 patients (13.7 %) were of their third or more gestations. For ultrasound examination we used a "middle" category standard ultrasound machine with 3.5 MHZ transabdominal transducer, after 1998 also 5.0 MHZ transvaginal transducer. The routine ultrasound examination was used in program of fetus state complete evaluation for detection the structural anomalies of fetus and after 1994 the abnormal values also were obtained by nuchal translucency measurement. The statistical analysis was performed by statistical package program for social sciences (SPSS).

**RESULTS:** Fetal abnormalities was found in 2016 cases (2.65 %) in first trimester of pregnancy. Overall detection rate, predictive positive value and specificity were respectively 52 %, 12 % and 72 %. Chromosomal abnormalities were 1890 (2.5 %), 1341 (71 %) were detected prenatally and 549 (29 %) – postnatally. Among prenatally diagnosed cases 917 (68 %) were subsequently detected in the second trimester because of false – negative results of first trimester.

**CONCLUSIONS:** Comparatively low rate of detected structural/chromosomal abnormalities of fetus in first trimester of pregnancy using exclusively routine first level ultrasound examination between 10 and 15 weeks of pregnancy was evident and now the success of prenatally detection of named pathology can be achievable only in case of using the multiple methods to be obligatory in daily clinical practice, preferably including in special diagnostic programmes.

## 121. DIAGNOSIS AND TREATMENT OF HAEMODINAMICALLY SIGNIFICANT FETAL TACHYCARDIA - REVIEW OF 33 CASES

### B. Pete, J. Hajdú, Z. Papp

I. Department of Obstetrics and Gynecology, Semmelweis University, Budapest, Hungary

**INTRODUCTION:** Fetal tachycardia may lead to an increased pre- and postnatal morbidity and mortality rate particularly if it is complicated by cardiac decompensation and hydrops fetalis.

**OBJECTIVE AND METHODS:** 33 cases of fetal tachycardia diagnosed and treated between 1993 and 2004 in the fetal echocardiography unit of our department and the data of postnatal care of the newborns delivered in our department from these pregnancies were reviewed.

**RESULTS:** The tachyarrhythmias were classified into atrial flutter (n=8), supraventricular tachycardia (n=18), arrhythmia absoluta (n=5), parasystolia (n=1) and brady-tachyarrhythmia (n=1). Six cases were complicated by hydrops fetalis, 13 cases by cardiac dysfunction. Transplacental antiarrhythmic therapy was applied in 22 cases, in 8 cases urgent cesarean section was done because of advanced gestational age, in 3 cases tachyarrhythmia resolved spontaneously or therapy was not indicated. The drug of first choice was digoxin, which was combined with amiodarone or verapamil (n=10). Transplacental therapy led to in utero cardioversion in 13/ 22 cases. The outcome of the 33 reviewed pregnancies was live birth in 27 cases, in utero death in 3 cases and 3 newborns were delivered elsewhere. The postnatal documentation of 24 newborns out of the 27 born in our department is available. At the time of birth 15/ 24 newborns were in sinus rhythm- out of whom 5 developed tachyarrhythmia later during the neonatal period-, 9/ 24 were tachycard. In the neonatal period 14 cases of tachyarrhythmia were detected altogether out of which 3 resolved spontaneously, in 7 cases antiarrhythmic therapy was successful, in 4 cases unsuccessful. In 2 of these latter cases electrical cardioversion led to sinus rhythm. Neurological disorder was not detected in any case. In the early postnatal period 2 in utero severely decompensated newborns died.

**CONCLUSIONS:** Survival and late prognosis of tachycard fetuses treated in utero is good. A prospective study of even more cases is required to establish uniform therapeutic guidelines and to provide appropriate follow-up data.

## 122. INVASIVE PRENATAL DIAGNOSTIC PROCEDURE DECISION-MAKING IN WOMEN UNDERGOING MULTIFETAL PREGNANCY REDUCTION

**L.P. Shulman**

Division of Reproductive Genetics, Department of Obstetrics and Gynecology, Northwestern University, Chicago, Illinois, USA

**OBJECTIVE:** Assess the prenatal diagnostic procedure decisions in women undergoing multifetal pregnancy reduction at increased risk for fetal aneuploidy.

**DESIGN AND METHODS:** Over the past 4 years, 22 women presented for multifetal pregnancy reduction ( $\geq 3$  fetuses) at increased risk for fetal aneuploidy ( $\geq 32$  years-old at EDC or other risks). All women were offered CVS prior to reduction or amniocentesis after reduction.

**RESULTS:** Four women (18.2%) chose to forego prenatal testing. Sixteen women (72.7%) chose to undergo CVS prior to reduction and 2 women (9.1%) chose post-reduction amniocentesis. Of the 16 women choosing CVS, a complete CVS analysis could not be accomplished in 2 cases because of placental location; post-reduction amniocentesis was offered and performed in both cases. There were no pregnancy losses in any of the 22 women; one chromosome abnormality (trisomy 21) was detected in the group undergoing CVS and in one of the 2 women who wished to undergo CVS but underwent amniocentesis because of placental location (trisomy 13).

**CONCLUSIONS:** Among women choosing to undergo multifetal reduction who are at increased risk for fetal chromosome abnormalities, the majority will likely select CVS, probably as result of this procedure being able to provide accurate diagnostic information prior to reduction. However, for patient-specific and procedural reasons, all women at increased risk for detectable prenatal abnormalities who have chosen to undergo multifetal reduction should be counseled about the availability of CVS and amniocentesis.

## 123. PLACENTAL ORIGIN OF THE EXTREME ELEVATION OF MATERNAL SERUM ALP LEVELS

**N. Than<sup>1,2</sup>, R. Magenheim<sup>1</sup>, A. Boronkai<sup>2</sup>, B. Hargitai<sup>1</sup>, P. Deres<sup>2</sup>, Sz. Bellyei<sup>2</sup>, A. Szigeti<sup>2</sup>, J. Rigó Jr.<sup>1</sup>, B. Sümegi<sup>2</sup>, Z. Papp<sup>1</sup>**

<sup>1</sup>I. Department of Obstetrics and Gynaecology, Semmelweis University, Budapest, HUNGARY

<sup>2</sup>Department of Biochemistry and Medical Chemistry, University of Pécs, HUNGARY

**OBJECTIVE:** Background of the rare diagnoses of high serum ALP levels (3609-9377 IU/l) in the third trimester of singleton and twin pregnancies were studied. Differential diagnostically important obstetrical, gynaecological and internal medical illnesses were ruled out, and possibilities of placental disorders were suggested. The goal was to reveal the functional and morphological changes in index placentas compared to controls, which led to the phenomenon.

**DESIGN AND METHODS:** ALP isoenzymes were determined from maternal serum samples using Hydragel Iso-Pal kit (Sebia). Histopathological examinations were performed on hematoxylin-eosine stained sections of index and control placentas. Immunohistochemical and Western-blot examinations were carried out on 5-5 samples of index and normal control placentas at the same gestational stage, using antibodies against placental ALP, Ki-67, phospho-Akt, phospho-p44/42 MAPK / Erk1/2, phospho-GSK-3, phospho-SAPK/JNK, total-Akt, total-GSK-3 and p38-MAPK.

**RESULTS:** Most of the total serum ALP revealed to be the P1 placental isoenzyme, which returned to the reference level in average postpartum at the 12<sup>th</sup> week. According to the histopathological examinations, there were intimal fibrin cushions in foetal chorionic vessels, groups of avascular villi in several foci throughout the index placental blocks and diffuse syncytiotrophoblast proliferation and syncytial knot formation on villous surfaces. ALP staining in the syncytiotrophoblast membrane of the index samples were much weaker and showed an interrupted pattern compared to the intensive continuous staining of the controls. Using anti-Ki-67 antibody, 8-10% of syncytiotrophoblast proliferation were found in samples of index placentas compared to the 1-2% of normal placentas. Elevated levels of protein kinases known to play important role in cell differentiation were also present in index placentas (152-561%) concerning the values of normal samples (100%). Based on immunohistochemical and molecular biological results, proliferation and differentiation rate of syncytiotrophoblasts were found to be 5 times more in the index samples than in control ones.

**CONCLUSIONS:** According to the histopathological, immunohistochemical and Western-blot examinations, the excessive amounts of ALP could be released by the immature brush border of newly formed syncytiotrophoblastic cells. Although healthy newborns were delivered, placental examinations are important and should be included in the differential diagnosis of pregnancies with extremely elevated ALP levels.

#### **124. HYPERTENSIVE DISEASE AND EVOLUTION OF PREGNANCY. A RETROSPECTIVE STUDY**

***M. Theodora, J. Papageorgiou, G. Daskalakis, A. Antsaklis***

High Risk Pregnancy Unit, I. Department of Obstetrics and Gynecology, University of Athens, Alexandra Hospital, Athens, GREECE

**OBJECTIVE:** The aim is the presentation of epidemiological characteristics and the evolution of pregnancy in cases with hypertensive state which were hospitalised in High Risk Pregnancy unit I. Department of Obstetrics and Gynecology, University of Athens, in "Alexandra Hospital".

**DESIGN AND METHODS:** 62 pregnant women who came to the hospital with hypertensive disease and were hospitalised from 1 /1 /2001 until 31 /12/2002 in High Risk Pregnancy unit. It is a retrospective study.

**RESULTS:** The mean age of the examined women was 31.5 years (22-53 years). 70% of them were nulliparas. Two of the cases were multiple pregnancies (twin). The mean of gestation age was 30± 6 weeks. Blood pressure at the time of admission was above 150/100 mmHg in 50% of the cases. 32% of our cases were already under treatment for hypertension, but with no result. In 30% of the cases proteinuria and/ or liver and renal disfunction were also present. The mean of hospitalization for adjusting blood pressure in normal were 8±2 days. Cesarean Section was the method of choice for delivery (98% of cases) and the mean of hospitalization after delivery were 7 days (two days in ICU).

**CONCLUSIONS:** Hypertensive states during pregnancy is serious complication resulting in high maternal and neonatal mortality and morbidity. Hospitalization in high risk pregnancy units is often necessary in order to adjust the medication and to manage those cases. Presence of liver and / or renal disfunction prolonged the duration of hospitalization for treatment and after delivery. Cesarean Section is the method of choice for delivery.

#### **125. PRELABOR RUPTURE OF MEMBRANES AND EVOLUTION OF PREGNANCY. A RETROSPECTIVE STUDY**

***M. Theodora, J. Papageorgiou, G. Daskalakis, A. Antsaklis***

High Risk Pregnancy Unit, I. Department of Obstetrics and Gynecology, University of Athens, " Alexandra Hospital", Athens, GREECE

**OBJECTIVE:** The aim is the presentation of epidemiological characteristics and the evolution of pregnancy in cases with Prelabor Rupture of Membranes (PROM) and Preterm Prelabour Rupture of Membranes (PPROM) which were hospitalised in High Risk Pregnancy Unit I. Department of Obstetrics and Gynecology University of Athens, in "Alexandra Hospital".

**DESIGN AND METHODS:** 145 pregnant women who came to the hospital with prelabor rupture of membranes and were hospitalised from 1 /1 /2001 until 31 /12/2002 in High Risk Pregnancy Unit. It is a retrospective study.

**RESULTS:** The mean age of the examined women was 27,5 years (15-42years). 63% of them were nulliparas. Five cases were multiple (twin) pregnancies. In 53% of the case prelabor rupture of membranes occurred before 37 completed weeks of gestation .The majority (83%) of the cases came to the hospital in less than 12 hours after PROM occurred. Onset of labor during the first 24 hour was noticed In 84% of the cases. 55% of deliveries were vaginal. When the rupture of membranes occurs before 24 completed weeks of gestation the perinatal outcome was extremely poor.

**CONCLUSIONS:** Onset of labour during the first 24 hour is the usual evolution of pregnancies complicated with prelabor rupture of membranes. There is no statistical difference in the roles of vaginal and operative delivery, although instrumental vaginal delivery was needed in some cases. Hospitalization and management of these pregnancies in high risk pregnancy units minimise the maternal and neonatal morbidity and mortality.

## **126. DIABETES MELLITUS AND EVOLUTION OF PREGNANCY. A RETROSPECTIVE STUDY**

**M. Theodora, J. Papageorgiou, G. Daskalakis, A. Antsaklis**

High Risk Pregnancy Unit, I. Department of Obstetrics and Gynecology, University of Athens, "Alexandra Hospital", Athens, GREECE

**OBJECTIVE:** The aim is the presentation of epidemiological characteristics and the evolution of pregnancy in cases complicated with diabetes mellitus which were hospitalised in High Risk Pregnancy Unit I. Department of Obstetrics and Gynecology University of Athens, in "Alexandra Hospital". 85 pregnant women who came to the hospital with diabetes mellitus type I and gestational diabetes and were hospitalised from 1 / 1 /2001 until 31 / 12/ 2002 in High Risk Pregnancy Unit. It is a retrospective study.

**RESULTS:** The mean age of women examined was 31.5 years (18-45 years). 62% of cases were regarding gestational diabetes. 44% of the cases were nulliparas. Two of the pregnancies were twin. The mean gestational age at first admission regarding cases of gestational diabetes was  $33 \pm 6$  weeks, whereas for cases regarding preexisting diabetes mellitus type I was smaller. The mean duration of hospitalisation was two (2) days for cases with GD and for cases with DM type I, was four (4) days. Cesarean section was the method of delivery in 70% of our cases.

**CONCLUSIONS:** Diabetes mellitus is a common complication of pregnancy. It is often necessary to hospitalise those women in high risk pregnancy units in order to control glucose levels in blood by continuous readjustment of insulin doses and patient reinforcement. Cesarean section was the method of delivery in 70% of our cases.

## **127. FETAL DACRYOCYSTOCELE: 3 CASES WITH SPONTANEOUS PRENATAL RESOLUTION**

**A. Wojakowski, L. Otano, D. Elías, F. Dovasio, G. Izbizky, H. Aiello, P. Farias**

Unit of Fetal Diagnosis and Treatment, Hospital Italiano de Buenos Aires, Buenos Aires, ARGENTINA

Dacryocystocele or lacrimal duct cyst is an obstruction of the lacrimal duct at the level of the cantonasal angle. It is a rare and benign condition in neonates and more than 90% of the cases regressed spontaneously within 6 month of life. However, the prevalence and the natural history in the antenatal period is poorly known.

**OBJECTIVE:** To present 3 cases of fetal dacryocystocele with spontaneous resolution before birth.

**DESIGN AND METHODS:** Observational. Routine obstetric ultrasound scan in a tertiary university hospital between 2001 and 2003 (6.000 births). One of the cases was referred from other centre.

**RESULTS:** All cases were detected during the third trimester (30 to 31 weeks). The ultrasound features were those of a cystic structure or hypoechoic mass, with a size between 4 and 9 mm, inferomedial to the orbit with no blood flow. Two were bilateral and one unilateral (right side). No other associated anomalies were detected. All were female fetuses and all evidenced spontaneous resolution of the dacryocystoceles before term births (34 to 36 weeks). Neonatal examinations were normal.

**DISCUSSION:** The spontaneous resolution of dacryocystocele "in utero" has been described incidentally. However, most reported cases with prenatal diagnosis persisted until the neonatal period. Typically, the diagnosis is made in the third trimester (after 27-28 weeks) when the canalization of the nasolacrimal duct should be completed. Differential diagnosis include hemangioma, anterior encephalocele, teratoma, glioma and rhabdomyosarcoma. The prenatal diagnosis of dacryocystocele would allow an appropriate neonatal management.

## **128. THE SITUATION OF PRENATAL DIAGNOSIS IN DIFFERENT CONTINENTS**

**J.M. Carrera**

Department of Obstetrics and Gynecology, Institut Universitari Dexeus, Barcelona, SPAIN

**OBJECTIVE:** The objective of this study is to review the situation of Prenatal Diagnosis (PD): ultrasonographic, biochemical and invasive PD in the five continents.

**DESIGN AND METHODS:** The information was obtained by means of the revision of the available bibliography and also of an inquiry made to PD experts all over the world.

**RESULTS:** The development of PD is very different depending on the place. The factor that have an influence on ratios of PN in a determinate are: 1) the technological capacity, 2) the financial situation (GNP), 3) the level of education and information of the population (GER), 4) the religious and/or cultural aspects, and 5) the legislation and state policy of several countries.

While Europe is doubtless the continent where PD has been much better developed, Africa has got problems much more serious than PD. The four apocalypses riders march on it: death, famine, war and pest (AIDS)

**CONCLUSIONS:** The PD is still patrimony of developed countries. Only a globalization of resources will make possible that every woman in every part of the world will have the rights to receive reliable information and access to PD.

## **129. FIRST TRIMESTER BIOCHEMICAL AND ULTRASOUND MARKER SCREENING - PRACTICAL ASPECTS**

**W. Holzgreve** (Basel, Switzerland): Abstract not received

## **130. MATERNAL-FETAL CONFLICTS. A PEDIATRICIAN PERSPECTIVE**

**L. Prudent** (Buenos Aires, Argentina): Abstract not received

## **131. AWARENESS AND ATTITUDE TOWARD PRENATAL DIAGNOSIS IN COUNTRIES WITHOUT LEGAL TERMINATION OF PREGNANCY**

**E.C. Gadow, H. Krupitzki**

Genetic Unit, Department of Obstetrics and Gynecology, CEMIC University Hospital, Genetic Group, Buenos Aires, ARGENTINA

**OBJECTIVE:** To determine the knowledge of, and the attitude and perception toward screening and prenatal diagnosis in pregnant women from countries where termination of pregnancy is not contemplated. To know the opinion of pregnant women about this last option, the impact of genetic counseling prior to the test, and the decision-making feelings. Understanding of primary prevention of neural tube defects (NTD) is also assessed.

**DESIGN AND METHODS:** Background population: In Latin America, prenatal diagnosis of chromosome abnormalities was first introduced in Argentina in 1971. Since then, several centers in this region started to perform this type of test. A pilot study was done through a semi quantitative questionnaire that patients were invited to complete after prenatal procedure. Since a significant bias was observed, a final protocol was implemented in order to allow parents to answer the questionnaire after counseling and before undergoing the study.

**RESULTS:** An unselected group of women belong to collaborative centers, one of which has performed approximately 16000 procedures during the last two decades. Ultrasound and biochemical screening began in 1994. Women receiving genetic counseling at least one week before the study were considered. The pilot study started in 2004 included 58 responses. The final protocol carried out until the time of abstract submission included 106 responses. Projection up to June will be approximately 350 responses.

**CONCLUSIONS:** Preliminary results showed a definite sociodemographic profile. Although most women knew that termination of pregnancy was not allowed, they were relieved by making the decision to undergo a prenatal study. As expected, the mean age of patients undergoing screening was lower. High educational level was reported in all cases. Although most women had knowledge of vitamins and folic acid, only few had taken them at the time of conception. To our knowledge, and except for one study addressed to a conspicuous malformation, this is the first psychosocial qualitative study of this type in the region.

### **132. INFORMED CHOICE: BRIDGING THE GAP BETWEEN POLICY AND PRACTICE**

**T. Marteau, E. Dormandy**

Psychology and Genetics Research Group, Kings College, London, UK

**OBJECTIVE:** To investigate whether lower uptake of antenatal Down syndrome screening in ethnic minority and socio-economically disadvantaged women reflect more negative attitudes towards undergoing the test or inconsistency between attitudes and uptake resulting in lower ratios of informed choice.

**DESIGN AND METHODS:** 1499 pregnant women offered prenatal screening of Down syndrome.

**RESULTS:** Uptake was higher in white and socio-economically advantaged women than in other women. There were no differences in attitudes between these groups towards undergoing the test: all women expressed relatively positive attitudes. Attitude-uptake consistency was higher in white and socio-economically advantaged women than others, particularly in those with positive attitudes towards undergoing the test (76% white women with positive attitudes had the test vs. 45% South Asian women (95% Confidence Interval of difference (95% CI diff) 18,43), and 78% socio-economically advantaged women vs. 62% disadvantaged women (95% CI diff 8,23)). Overall rates of informed choice were higher for white and socio-economically advantaged women (56% of white vs. 20% of South Asian women (95% CI diff 28,44); 59% of socio-economically advantaged vs. 41% of disadvantaged women (95% CI diff 3,24)).

**CONCLUSIONS:** Lower uptake of Down syndrome screening in ethnic minority and socio-economically disadvantaged women does not reflect more negative attitudes towards screening but rather lower rates of informed choice. UK healthcare systems appear to facilitate informed choices in the context of antenatal Down syndrome screening less well for ethnic minority and socio-economically disadvantaged women than other women. Ways of facilitating informed choice in these latter groups will be presented.

### **133. PARENTAL DECISION TO ABORT OR CONTINUE A PREGNANCY WITH CYTOGENETIC ABNORMAL FINDING AFTER AN INVASIVE PRENATAL TEST**

**R. Quadrelli, A. Vaglio**

Instituto de Genética Médica, Hospital Italiano, Montevideo, URUGUAY

**INTRODUCTION:** Our Institute of Medical Genetics covers different areas of Genetics through genetic diagnosis and counselling. One of these areas is prenatal diagnosis. Patients from different parts of the country are referred by their obstetricians for consultation, genetic counselling and prenatal diagnosis techniques. This procedure makes it difficult to perform follow-up studies.

**OBJECTIVES:** The objective of this study was to confirm the significance of genetic counselling as a main factor in the decision-making process to continue, or not, pregnancy in patients with an abnormal chromosomal prenatal diagnosis.

**DESIGN AND METHODS:** Out of 17,396 chromosomal prenatal diagnoses made between 1982 and 2003 in chorionic villus (2,740) and amniotic fluid (14,656), a retrospective study was done about the decision to discontinue, or not, pregnancy after an abnormal prenatal diagnosis made in 376 of these cases. Out of 376 detected anomalies, we obtained information from 232 (61.7%). Other 5 factors were also assessed: maternal age, gestational age, presence of other children, ultrasonographic findings and type of anomaly according to its clinical significance.

Chromosomal abnormalities were divided into five categories: Down's syndrome, which was analysed separately, since it is the most frequent chromosomal abnormality, familiar to the population due to its clinical significance; other unbalanced autosomal abnormalities, sex chromosomal abnormalities, de novo markers and balanced de novo chromosomal abnormalities.

**RESULTS:** We found that 81% of trisomy 21 cases were discontinued.

Among the other chromosomal abnormalities, termination was associated with genetic counselling. The only significant variable in couples' behaviour was the type of anomaly and its clinical significance. Regarding the other variables herein analysed, no marked differences were observed in the decision-making process.

**CONCLUSIONS:** Genetic counselling plays a major role in couples' decision-making, since it allows to inform the clinical significance of chromosomal abnormalities detected before birth. In our country, the lack of a legal frame for the voluntary termination of pregnancy does not prevent couples from undergoing this procedure.

## **134. FETAL LEARNING**

### ***H. Nakano***

Department of Obstetrics and Gynecology, Graduate School of Medical Sciences, Kyushu University, JAPAN

An observation of fetal behavior shows us anatomical and functional development of the fetal brain. From the viewpoint that the fetus is existing in an environment relatively free of external physiological and social influences, we can hypothesized that the fetal pacemaker must be organized at a basic level during intrauterine life. In this regard, the fetal behavior might be considered as an expression of intrinsic CNS function. However, two issues are to come appear. One is, "Is the fetus in the free running system?" Another one is, "Then, can we evaluate and estimate the cortical development" For many years, it was thought that the newborn infant did not possess a functioning memory, but rather, memory in itself developed over the months and years following birth. The study of newborns and premature infants in recent years, however, has changed this view. Newborns have been shown, via a variety of learning paradigms, such as habituation, classical conditioning, associative learning and imitation, to possess a functioning memory. A memory begins prenatally and the period of birth merely marks a transition from in utero memory function to ex utero. In this context, we might not be able to hypothesize that the fetal behavior is an expression of intrinsic CNS function.

Learning is believed to occur in the absence of overt behavior just after stimulation, but actual occurrence can only be inferred from changes in behavior. When a sensory organ receives a stimulus of any kind, it sends some signals in the sensory system in the brain, leading to an activation of related areas including learning centers as well as the memory center. After these centers determine the intensity and type of response, they send a signal to the motor system, resulting in a response such as movement, secretion etc.

With this theoretical conception in mind, learning might be considered to reflect more integrated brain function and the cortical development should be involved in learning process.

Such will be the main issue to be discussed at the coming opportunity.

## **135. CLINICAL GENETICS AND MATERNAL-FETAL MEDICINE: LESSONS FROM THE LAW**

### ***A. Milunsky***

Center for Human Genetics, Boston University School of Medicine, Boston, MA, USA

Medical negligence is a universal problem that needs to be dealt with in all countries. Medical malpractice is governed by an acknowledged "Standard of Care" in the USA and most western countries. Where malpractice is claimed, physicians are judged by a standard requiring what a prudent and reasonable physician would have done under similar circumstances. A breach of that standard associated with a duty to perform that results in damage to a patient is regarded as malpractice. In the face of enormous advances in human genetics, maternal-fetal medicine practice has become more demanding. At least in the USA many lawsuits have arisen due to a failure of the obstetrician to refer (or confer) for genetic counseling or evaluation. Consequently, these lawsuits have focused on issues that include failure to make a precise genetic diagnosis, carrier determination, the offer or provision of maternal serum screening or predictive tests or prenatal diagnosis. Many of these claims are advanced on the basis of a doctrine of "loss of chance". This doctrine posits that the failure to have been informed, tested, diagnosed or treated, deprived the parent (for example) from having a child unaffected by a specified disorder, or deprived a child being born unaffected, having no opportunity to avoid a fatal disorder, to obtain timely treatment, or even to have been born at all!

Litigation about the diagnosis and management of genetic disorders can be considered in the following categories (1) Preconception care failures (2) Pregnancy care failures (3) Failures in genetic counseling (4) Failures in communication (5) Laboratory failures (6) Failures in documentation.

Professional societies have the responsibility of issuing frequent technical bulletins that draw their members' attention to guidelines and standards of care in maternal-fetal medicine and other specialty practices.

### **136. CHANGE IN PUBLIC DEMAND FOR GENETIC COUNSELING IN THE PAST 30 YEARS**

**Z. Papp**

I. Department of Obstetrics and Gynecology, Semmelweis University, Budapest, HUNGARY

Genetic counseling is a field of professional expertise that involves diagnosis, provision of information and consultation with individuals about their genetic makeup and chances bearing a child with severe congenital defect. Genetic counseling units usually headed by medical geneticists and are located in university hospitals or medical centers.

I established a genetic counseling service within the Debrecen University obstetric department in 1966. During the first 10 years new diagnostic techniques applicable in pregnancy such as amniocentesis and chromosome analysis from amniotic fluid cells were introduced and the program was officially confirmed in 1976. Since then, the service has become the main prenatal genetic counseling unit in Hungary. This project was also established in Budapest in 1990. Data from the first 15 years (1976-1990) were collected in Debrecen, and from the last decade of this period (1991-2002), in Budapest.

In the present study, experiences from the last three decades (1976-2002) have been collected, and in order to demonstrate the changing demands, differentiated into five 5-year subgroups. During these almost 30 years over 55.000 couples were counseled regarding almost 60.000 pregnancies and counseling was received before planned pregnancies in over 70.000 counseling situations. The number of couples requesting genetic counsel has continuously increased since the establishment of our service. The distribution of genetic diseases or congenital anomalies as indicators for counseling is influenced mainly by the continuous improvement of pre- and postnatal imaging, ultrasound techniques and the availability of molecular genetic methods. When prenatal diagnosis was not available, the reproductive decision was strongly influenced by a 25-50% genetic risk of disease in a future offspring. When prenatal diagnosis was available, only 15% of couples asked for termination of pregnancy or did not attempt a pregnancy. The majority of women (85%) decided to continue or attempt a pregnancy, and nearly 70% of them requested prenatal diagnosis.

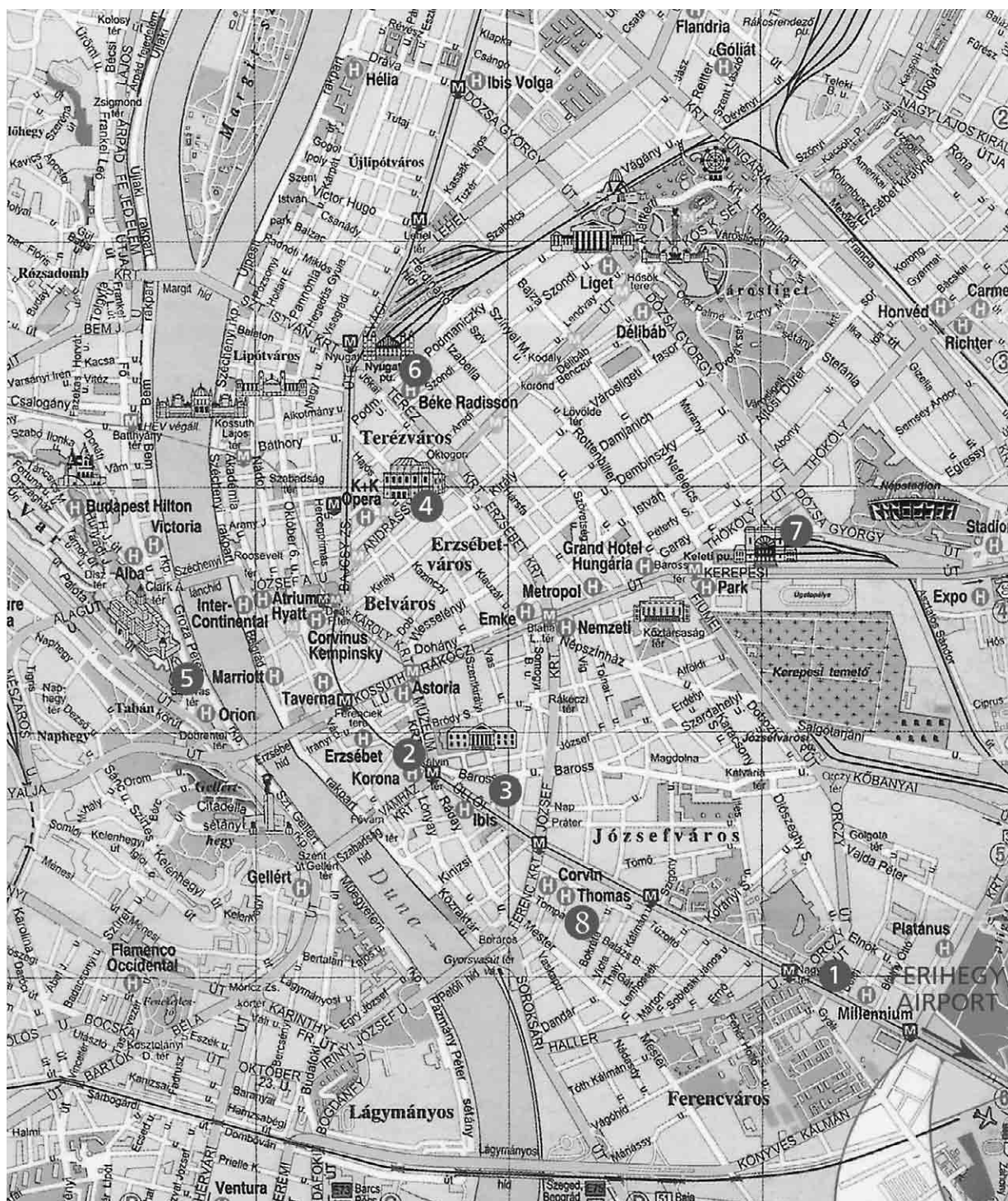
Predictive testing represents a new and growing field of medical tests. The public demand for them is increasing, but the adequate counseling is not always provided with the results. Genetic counseling prior and after predictive testing is essential especially when couples ask for prenatal or preimplantation genetic diagnosis.

# AUTHOR INDEX

The numbers referred to in this index are the abstract numbers. Bold numbers indicate the presenter

- Adinolfi M: 26  
Aguayo A: 81  
Aiello H: **61**, 80, 127  
Alberman E: 69  
Albrechts JCM: 65  
Amit A: 13  
Amorocho B: 12  
Antsaklis A: 31, **60**, 115, 124, 125, 126  
Antsaklis P: 31  
Argibay P: **98**, 107  
Azem F: 13  
Ballesteros A: 12, 16  
Balog J: 46  
Bán Z: 24, 29, 32, 43, 46, **55**, 62, 79, 90, 103, 114  
Barakonyi E: 62  
Barbich M: 98, 107  
Beke A: 29, 32, 43, **62**, 63, 90, 109  
Belics Z: 62, **63**  
Bellyei Sz: 123  
Benkő R: 43  
Ben Yosef D: 13  
Bermudez M: 7  
Bianchi DW: **18**  
Bilardo CM: 86  
Bindra R: 25  
Bischoff FZ: **19**  
Bishop A: 74  
Blanchet P: 72  
Blankenstein MA: 23  
Bóna A: 43  
Borobio V: 82, 102  
Boronkai Á: 123  
Borrell A: 44, 52, **58**, 76, 82, 102  
Botet F: 102  
Boubli L: 30  
Bouman K: 88  
Bóze T: 117  
Brestak M: 87  
Brioschi PA: 83  
Brouckova M: 87  
Buendía P: 17  
Bulmer J: 26  
Bush M: 118  
Canick J: **53**  
Cararach V: 76, 82, 102  
Carlin J: 74  
Carrera JM: **128**  
Casals E: 52, 76  
Chan AMY: 91  
Chan KCA: 25  
Chasen ST: **59**  
Chaudet H: 30  
Chaze A-M: 72  
Chervenak FA: 59  
Cheung MC: 20  
Chim SSC: **25**  
Chitayat D: **64**  
Chitty LS: **56**, 74, 119  
Chiu RWK: 25  
Chmura M: 67  
Chu L: 7  
Chui DHK: 28  
Cieslak J: 2  
Cioni R: 26  
Cirigliano V: **26**  
Claustres M: 72  
Cobo T: **102**  
Cohen J: 5, 7  
Coll O: 44  
Colls P: 5  
Conn CM: 11  
Costa C: 26  
Craft I: 10  
Cram D: 27  
Creemers J: 78  
Cronister A: 45  
Csaba Á: 62, **103**  
Csabay L: 63  
Csikós M: **43**  
Cuckle H: 58  
Cullinane F: 74  
Cupisti S: 11  
Cusi V: 81  
Daphnis D: **10**  
Daskalakis G: 124, 125, 126  
De Clippel KAJ: **104**  
Deckindt C: 112  
De Leeuw N: 78  
Delhanty JDA: 10, 11  
De Pater J: **65**, 77  
D'Ercole C: 30  
Deres P: 123  
Dévényi N: 117  
De Vries B: 78  
Dienes J: **105**  
Dijkhuis J: 88  
Dijkhuizen T: 88  
Ditesheim PJ: 83  
Diaz A: 47  
DiMaio M: 45  
Donnenfeld A: 45  
Dorland M: 65  
Dormandy E: 132  
Dovasio F: 127  
Eder M: 99  
Elias D: 98, 127  
Elias P: 111  
El-Sheikhah A: 25  
Elvers LH: 85  
Engelen JJM: 65  
Escudero T: 5  
Evans M: **94**  
Faas B: **66**  
Faed MJW: 11  
Farias P: 98, 107, 127  
Farooq T: 9  
Farre MT: 82  
Fast C: 99  
Faway CH: 83  
Fekete T: 62, 63  
Feng D: 20  
Ferraretti AP: 4  
Fiddler M: 38  
Figuera F: 82  
Fiorentino F: **34**  
Fischer J: 5  
Fisk N: **97**  
Fortuny A: **52**, 76, 82  
Fragouli E: **11**  
Frumkin Z: 13  
Gabert J: 30  
Gadow EC: 61, 80, **131**  
Garamvölgyi Z: **106**, 117  
Garcia M: **107**  
Garda AL: **12**  
Gardó S: 118  
Gerssen-Schoorl KBJ: 88  
Geurts van Kessel A: 66  
Geyer J: 10  
Gianaroli L: **4**  
Ginsberg N: **67**  
Go ATJJ: 23  
Gómez E: 12  
Goncé A: 76  
Groenendijk BCW: 95  
Groenewout M: **108**  
Guyon F: 112  
Haas J: 99  
Hafner T: 49  
Halmos A: 63  
Hajdú J: **109**, 121  
Hajdú K: 50  
Hajek P: 75  
Hallam S: 45  
Halliday J: **68**, 74  
Hamel B: 66  
Hammond C: 48  
Hargitai B: 123  
Harmath Á: 24, **110**  
Harper J: **8**, 10  
Havlovicova M: 75  
Herczegfalvi Á: 50  
Hernandez SS: **44**  
Hidalgo A: 107  
Hidvégi J: 106  
Hladikova M: 75  
Hodík K: **111**  
Hogg M: 25  
Hollmann C: 33  
Holzgreve W: **129**  
Horn S: 9  
Horovitz J: **112**  
Horváth A: 43  
Horváth R: 46  
Hruby E: 109, **113**  
Huang T: 64, **69**, **70**, 93  
Hultén M: **22**  
Hyon SH: 98, 107  
Igarzábal L: 61, 80  
Illkevitch Y: 2  
Izbizky G: 127  
Jaffe S: 9  
Jerkovic S: 10  
Jewelwicz R: 9  
Johnson KL: 18  
Joó JG: 62, 90, 103, **114**  
Kan YW: **20**  
Kanavakis E: 31  
Karcagi V: 46, 50  
Kárpáti S: 43  
Katsoulis I: **115**  
Katz-Jaffe M: **27**  
Kavalakis Y: 31  
Kékes K: 105  
Khatamee M: **9**  
Khosrotehrani K: 18  
Kirilova I: 2, 92  
Kleijer W: **35**  
Knekt AC: 86  
Kolialexi A: 31  
Kondo Y: 89  
Kooper A: 66  
Kos M: 49  
Kovács Z: **116**  
Kovalev S: 92  
Krapiva G: 92  
Krasznai I: 106, **117**  
Kroes HY: 65  
Krupitzki H: 131  
Kuliev A: **2**, 3  
Kurjak A: 39  
Kwok YK: 28  
Lallaoui H: 72  
Lam YH: 28, 71  
Landeras J: 12, 16  
Lapinski R: 118  
Larrabee PB: 18  
Larvandaburn M: 47

Lau ET: **28**, 73  
 Lau TK: 25  
 Lázár L: **24**, **29**, 79, 103  
 Lee CP: **28**, **71**  
 Lefort G: **72**  
 Lemes A: 47  
 Lessing JB: 13  
 Leung KY: 28  
 Leung TN: 25  
 Leung WC: **73**  
 Levy-Mozziconacci A: **30**  
 Lewis S: **74**  
 Lo YMD: **21**, 25  
 Lochu P: 72  
 Loeber JG: 85  
 Lorenti A: 98  
 Louckova M: 87  
 Luo HY: 28  
 Macek M: **75**  
 Madan K: 77  
 Magenheim R: 123  
 Magli MC: 4  
 Mahoney M: **45**  
 Malcov M: **13**  
 Mancini J: 30  
 Mangione R: 112  
 Mantzaris D: 27  
 Maria O: 5  
 Marteau T: 74, **132**  
 Martín J: **14**  
 Martínez MA: **76**  
 Martínez MC: 12  
 Martínez S: 12  
 Martinez-Crespo JA: 102  
 Martinez-Zamora MA: 102  
 Marton T: 109  
 Matayoshi T: 80  
 Mateu E: **15**, 16, 17  
 Mavrou A: **31**  
 Mayer P: 46  
 McCulloch LB: 59  
 Meier C: 70, 93  
 Mercadé I: 76  
 Mercader A: 14, 16, 17  
 Métneki J: 84  
 Mey-Raz N: 13  
 Mok KM: 91  
 Mills JA: 11  
 Milunsky A: **135**  
 Muggli E: 68  
 Mulders MAM: 23  
 Muller FF: 30  
 Muller MA: 86  
 Munne S: **5**, 7  
 Musilova I: 111  
 Nadal A: 102  
 Nagy B: 29  
 Nagy G: 105  
 Nagy GyR: **32**, 79  
 Nagy S: **118**  
 Nakano H: **134**  
 Natekova J: 111  
 Ng EKO: 25  
 Nguyen The H: 83  
 Nieuwint AWM: **77**  
 Nikolaidis KH: 25  
 Nikolini U: **41**  
 Novikova I: 92  
 Old J: **36**  
 Olde Weghuis D: **78**  
 Oroszné Nagy J: 32, **79**  
 Otano L: **37**, 61, **80**, 127  
 Oudejans CBM: **23**  
 Owolabi T: 70, 93  
 Pajkrt E: **119**  
 Papageorgiou I: 115  
 Papageorgiou J: 124, 125, 126  
 Papantoniou N: 115  
 Papatashvili A: **120**  
 Papp Cs: 62, 90, 114  
 Papp Z: 24, 29, 32, 43, 62, 63, 79, 103, 109, 114, 121, 123, **136**  
 Pellestor F: 72  
 Pellicer A: 14, 15, 16, 17  
 Pérez I: 12, **16**  
 Perez Iribar MM: **81**  
 Pérez M: **82**  
 Pergament E: **38**  
 Pertl B: **99**  
 Pescia G: **83**  
 Pete B: 109, **121**  
 Philip J: **40**  
 Pikó H: **46**  
 Pilalis A: 31  
 Pinton A: 72  
 Pisa S: 44  
 Podholova M: 111  
 Poelmann RE: 95  
 Ponceillé B: 30  
 Potuznikova P: 75  
 Pribushenya O: 92  
 Prudent L: **130**  
 Puerto B: 82, 102  
 Quadrelli R: 47, 133  
 Quennan J: **96**  
 Rácz E: 43  
 Rechitsky S: 3  
 Remohí J: 14, 15, 16, 17  
 Resch B: 99  
 Rigó J Jr: 29, 106, 116, 117, 123  
 Rodeck C: **101**  
 Rodrigo L: 15, **17**  
 Roux F: 30  
 Rubio C: 14, 15, 16, 17  
 Rukavina-Stavljenic A: 49  
 Sándor J: **84**  
 Sarda P: 72  
 Saura R: 112  
 Savenko L: 92  
 Schielen PCJI: **85**  
 Schmid D: 83  
 Schwartz T: 13  
 Sepulveda W: **100**  
 Sermon K: **6**  
 Serrano C: 15  
 Sertic J: 49  
 Shreder S: 92  
 Shulman LP: **48**, 67, **122**  
 Sibul M: 67  
 Siffel Cs: 84  
 Sikkema-Raddatz B: 88  
 Simandlova M: 75  
 Simón C: 14  
 Simpson JL: **1**  
 Sipos F: 32  
 Siska É: 50  
 Sistermans E: 78  
 Smits A: 66  
 Snijder S: **86**  
 Sole F: 26  
 Sonta S: 89  
 Souka A: 31  
 Suijkerbuijk RF: **88**  
 Sümegi B: 123  
 Summers AM: 64, 69, 70, 93  
 Suzumori K: **89**  
 Stachelhaus S: 33  
 Steegers EAP: 104, 108  
 Stejskal D: **87**  
 Stekelenburg-de Vos S: 95  
 Steuerwald N: 7  
 Stipoljev F: **39**, **49**  
 Struijk PC: 104, 108  
 Sugiura M: 89  
 Suy A: 44  
 Szabó I: 63  
 Szendei Gy: 117  
 Szigeti A: 123  
 Szigeti Zs: **90**, 114  
 Szunyogh M: 84  
 Tanemura M: 89  
 Tang M: 71  
 Tang MHY: 28, 73  
 Tang R: 71  
 Tarnawa V: **50**  
 Than N: **123**  
 Theodora M: **124**, **125**, **126**  
 Tímár L: 50  
 To WK: 71, **91**  
 Tong Y: 25  
 Tóth A: 50  
 Tóth-Pál E: 62, 90, 114  
 Tse HY: 71  
 Tsui NBY: 25  
 Tsukerman G: 67, **92**  
 Urlesberger B: 99  
 Ursem NTC: 95  
 Vaglio A: **47**, **133**  
 Vago P: 72  
 Van den Berg P: 66  
 Van der Veen AZ: 88  
 Van Oppen C: 65  
 Van Ravenswaay C: 66, 78  
 Vanrell JA: 102  
 Van Vugt JMG: 23  
 Vela A: 81  
 Venchikova N: 92  
 Verjaal M: 86  
 Verschuren M: 65  
 Verlinsky Y: 2, **3**, 67  
 Vieiro M: 107  
 Vilimova S: 75  
 Ville Y: **42**  
 Visser A: 23  
 Vlk R: 75  
 Wald N: **54**, 69  
 Wapner R: **51**  
 Wataganara T: 18  
 Weier U: 7  
 Wells D: **7**, 11  
 Weseley A: 9  
 Whaley K: 11  
 Wijman MJNC: 104, 108  
 Wladimiroff JW: **95**, 104, 108  
 Wojakovski A: **127**  
 Wolstenholme J: **57**  
 Wong K: 71  
 Wong SF: 71  
 Woo H: 71  
 Wyatt PR: 70, **93**  
 Yaron Y: 13  
 Yates C: 48  
 Zabala T: 81  
 Zheng X: 5  
 Zimmermann S: **33**  
 Zlatopolsky Z: 2



- |  |   |
|--|---|
| 1. Congress venue  | 5. Semmelweis Museum of Medical History |
| 2. Hotel Korona  | 6. Western Railway Station              |
| 3. I. University Department of Obstetrics and Gynecology | 7. Eastern Railway Station              |
| 4. Opera House   | 8. Hotel Thomas                         |