11.00–11.15

O-100. The administration of the GnRH antagonist cetrorelix to oocyte donors simplifies oocyte donation

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Aim: To evaluate the efficacy of the GnRH antagonist cetrorelix and recombinant FSH, gonal F, for controlled ovarian stimulation in a oocyte donor programme.

Material and methods: Six oocyte donors were commenced on gonal F (150 IU) and two on gonal F 225 IU daily on day 4 of menses. Subcutaneous cetrorelix 0.25 mg was commenced on day 8 and both drugs continued until the day of administration of HCG. Six recipients who were premenopausal were down-regulated with intranasal nafarelin 400 mcg twice daily and this was discontinued 2 days prior to embryo transfer. The remaining two recipients who had premature menopause did not require down-regulation. The recipients received a step-up dose of oral estradiol valerate (EV) daily commencing on day 1 of the donor cycle (EV 2 mg days 1–5, EV 4 mg days 6–10, EV 6 mg day 11) to day of donor's oocyte retrieval. EV 4 mg was continued until a pregnancy test 14 days later. They also received i.m. gestone (50 mg) daily from day of donor's oocyte retrieval for two consecutive days. Following this, i.m. gestone (100 mg) daily or progesterone vaginal suppositories (cyclogest 200 mg twice daily) were administered until the pregnancy test. For women who conceived, EV 8 mg daily and cyclogest 200 mg twice daily were continued until 9 weeks gestation.

Results: The median number (range) of oocytes retrieved was 7 (3–13). None of the donors had a premature LH surge or complications post-operatively. With the exception of a recipient who had failed fertilization, the remaining recipients had two embryos transferred. The median (range) number of days of ovarian stimulation, number of gonal F ampoules administered and days of cetrorelix administered were 9 (7–12), 18 (14–24) and 5 days (3–8) respectively. The clinical pregnancy rate per cycle was 50% (4/8) and one of these women miscarried at 8 weeks gestation. Three women (37.3%) had term deliveries. One woman had a biochemical pregnancy.

Conclusions: This small study using a combination of cetrorelix and gonal F resulted in a high pregnancy rate, shortened the duration of treatment for the donor and provided optimal synchronization of donor–recipient cycles.

11.15–11.30

O-101. GnRH antagonist on alternate days in an IVF/ICSI programme can suppress premature ovulation

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Intoduction: GnRH antagonists produce an immediate suppression in endogenous gonadotrophin secretion. Protocols using the GnRH antagonist are based on either a single dose of 3 mg or a multiple dose regime with a lower dose, 0.25 mg daily, which begins on day 6 of the cycle and continues until HCG administration. In this study we followed a multiple dose protocol but GnRH antagonist was administered on alternate days. The aim of this study was to evaluate this regime in preventing premature ovulation and LH surge.

Materials and methods: This was a prospective study, in which patients undergoing IVF/ICSI treatment cycles were recruited. Thirty-four women had completed their treatment cycles. All patients had ovulation stimulation with gonadotrophins starting day 2 or 3 of the cycle. GnRH antagonist 0.25 mg/day on alternate days was started when the leading follicle was ⩾14 mm. Urinary LH testing was performed twice a day once the antagonist was commenced. HCG was administered when at least three follicles reached an average diameter of 18 mm.

Results: The mean number of GnRH antagonist 0.25 mg ampoules used was 2.9 ± 1.2 (range 1–6). Three cycles were abandoned due to low response. The mean age of the patients was 33.6 ± 4.2 years (range 26–40) and the mean early follicular phase serum FSH was 8.4 ± 2.9 mIU/ml (range 4.1–16.1). Mean number of oocytes collected was 11.1 ± 5.7 (range 3–31). The fertilization rate was 51.6%. The mean number of embryos transferred was 2.1 ± 0.68 (ranges 1–3). Six women had singleton pregnancy (17.6%), yet four miscarried. Two of the patients (5.8%) had premature ovulation as confirmed by ultasonographic findings in both of them with positive urine LH surge testing in one woman.

Conclusions: This study showed that using GnRH antagonist 0.25 mg on alternate days, during ovarian stimulation for IVF or ICSI treatment resulted in 5.8% ovulation. Ovulation was seen in low responders requiring higher doses of gonadotrophins. Both patients were low responders requiring an increase in gonadotrophin dose to 450 IU; one and three follicles developed respectively.

ART – ICSI

Tuesday 2 July 2002
Hall D

10.00–10.15

O-102. Selection of sperm with low aneuploidy frequencies for ICSI

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Introduction: We have previously shown a close correlation between the proportion of immature sperm with cytoplasmic retention and frequency of chromosomal aneuploidies. This relationship is based on the dual role of the HspA2 chaperone, which supports meiosis as a component of the synaptonemal complex, and facilitates plasma membrane remodelling and the formation of the zona pellucida and hyaluronic acid (HA) binding sites during spermiogenesis. The increased rate of chromosomal aberrations and other potential consequences of using immature sperm for ICSI is of major concern. We present a new approach for selection of individual mature sperm with decreased frequency of chromosomal aberrations. The method utilizes sperm binding to solid state HA. HA is a normally occurring component of the female reproductive tract.

Materials and methods: Washed sperm of 12 moderately oligozoospermic men (OS, sperm concentration, mean ± SEM: 20.6 ± 1.7×10^6/ml; motility: 54.1 ± 2.5%) and 80% isolate gradient sperm pellets from 12 normospermic IVF patients (ISL80, sperm concentration: 118 ± 21.4×10^6/ml, motility: 59.1 ± 4.9%) were studied. Sperm suspended in human tubal fluid were placed over HA spots bonded to Petri dishes. After incubation for 15 min, the HA-attached sperm were collected using an ICSI micropipette. Aliquots of the sperm suspension and HA-bound sperm were examined after fluorescent in-situ hybridization, using centromeric probes for the X, Y and 17 chromosomes. Data were examined by χ^2-analysis.

Results: In each man we analysed the initial sperm suspension (mean: 4500 sperm, 110 000 sperm in the 24 men), and all HA-bound sperm collected in the 12 OS men (mean: 753, range: 224–1142) and 12 ISL80 (mean: 954, range 360–1955). In both the OS and ISL80 groups, the disomy rates declined in the HA-bound fractions (Table). The decrease for sex chromosomes was ~4-fold, even though the ISL80 samples were 80% isolate pellets of normozoospermic men (thus 'ideal sperm'). Diploid sperm decreased 6-fold in both groups ($P < 0.001$).

	Group OS			Group ISL90		
	Disomy		Diploidy	Disomy		Diploidy
	Sex	17		Sex	17	
Initial (%)	0.35	0.23	0.81	0.25	0.12	0.58
HA-bound (%)	0.09	0.04	0.13	0.06	0.07	0.10
Reduction	×4.0	×5.3	×6.1	×4.1	×1.7	×6.1
P-value (χ^2)	< 0.001	< 0.001	< 0.001	< 0.001	NS	< 0.001

NS = not significant.

Conclusions: HA selection eliminated sperm with disomy and diploidy. The 4-fold decline of sex chromosome disomies is consistent with the increase of chromosomal aberrations in ICSI children. In spite of the sample differences, the aneuploidy and diploidy rates in the HA-bound fraction declined to a narrow 0.04–0.13% range. Thus, HA sperm selection provides a new, safe and efficient solution for selection of mature sperm for ICSI.

10.15–10.30

O-103. Controlled comparison of ICSI and laser-assisted ICSI in low responder patients

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Introduction: High fertilization rates can be obtained with the ICSI technique. Nevertheless, some oocytes may be damaged during the procedure. Oocyte survival is often compromised by abnormal oolemma breakage patterns during the penetration of the microinjection needle. This situation could be related to the ovarian stimulation response. We have recently suggested that oocyte damage can be reduced by performing ICSI through a laser-drilled hole in the zona pellucida. This new approach appeared to be suitable for patients whose oocytes show inherent fragility and high degeneration rates after the standard ICSI procedure. The aim of this study was to determine if laser-assisted ICSI may also improve the outcome in low responder patients where only few oocytes can be obtained.

Materials and methods: Thirty patients were randomly allocated on the day of HCG administration into two groups for IVF technique: standard ICSI and laser-assisted ICSI. The inclusion criteria were female age <40 years and seven or less follicles obtained after controlled ovarian hyperstimulation.

Results: The results are displayed in the table.

	ICSI group	Laser-assisted ICSI group	*P*-value
Cycles	15	15	
Female age (±SD)	35.7 ± 1.5	36.1 ± 1.4	NS
No. FSH ampoules (±SD)	70.5 ± 14.5	72.3 ± 15	NS
Day of stimulation (±SD)	13.1 ± 0.9	13 ± 0.8	NS
MII oocytes per patients (±SD)	4.1 ± 1.6	3.9 ± 1.5	NS
Fertilization rate per injected oocytes (%)	41/61 (67.2)	51/59 (86.4)	< 0.01
Cleavage rate	37/41 (90.2)	47/51 (92.1)	NS
No. embryo transfers performed (%)	11 (73.3)	15 (100)	< 0.05
Clinical pregnancy rate per cycle (%)	3 (20.0)	7 (46.6)	< 0.05
Implantation rate (%)	4/37 (10.8)	7/47 (14.9)	NS

NS = not significant.

Conclusions: Laser-assisted ICSI statistically increases the fertilization rate in a population of patients where very few oocytes can be obtained after aggressive hyperstimulation treatments. The higher pregnancy rate per cycle observed in the study group is due to a higher number of embryo transfers performed. We suggest that a poor response to ovarian hyperstimulation might represent an indication for laser-assisted ICSI technique.

10.30–10.45

O-104. Chromosomal abnormalities in embryos derived from testicular sperm extraction

Silber S.J.[1], Sadowy S.[2], Lenahan K.[1], Kilani Z.[3], Gianaroli L.[4] and Munne S.[2]

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Introduction: There is considerable concern about the effect of severe spermatogenic defects on the health of offspring derived from ICSI. We sought to compare the rate of chromosome abnormalities in embryos obtained from patients with non-obstructive azoospermia undergoing testicular sperm extraction (TESE) with embryos derived from patients undergoing ICSI with ejaculated sperm.

Materials and methods: Preimplantation genetic diagnosis (PGD) was performed in patients undergoing ICSI for oligospermia and in patients undergoing TESE. Chromosome enumeration was performed by fluorescence in-situ hybridization using 5–8 probes on one biopsied blastomere. Embryos classified as abnormal were then reanalysed to study mosaicism and to assess the reliability of the single cell determination.

Results: There was a higher rate of chaotic mosaicism in embryos derived from TESE–ICSI than in those derived from ICSI with ejaculated sperm. Of 1173 embryos in the ICSI group, 45.8% of embryos were normal, 23.9% aneuploid, 25.2% mosaic, 4.2% polyploid and 2.7% haploid. In contrast, of the 93 embryos analysed from the TESE group, only 25.8% of embryos were normal, 15.1% aneuploid, 51.6% mosaic, 5.4% polyploid and 2.2% haploid. The differences between the rate of normal embryos and mosaic embryos in the two groups were highly significant ($P < 0.0001$). No statistically significant differences in aneuploidy were observed. Furthermore, the rates of aneuploidy per chromosome were similar in both groups. Embryos were classified as mosaic based on single cell analysis if more than two chromosomes exhibited an abnormal number (but the embryo was neither haploid nor polyploid). Of 22 TESE embryos diagnosed as mosaic by PGD and reanalysed, 21 (95.4%) were found to be chaotic mosaics. Of 130 control embryos diagnosed as mosaic by PGD, 80 (61.5%) were verified to be chaotic mosaics. The remaining reanalysed mosaics were either diploid/polyploid, diploid/haploid, diploid/aneuploid or other more rare combinations.

Conclusions: These preliminary results suggest a high incidence of mosaicism in embryos derived from TESE. Because the male centrosome is the organizing centre for the first mitotic spindle, a sperm abnormality may produce chaotic mosaicism in subsequent cleaving embryos.

10.45–11.00

O-105. Impact of ICSI on embryo freezability: a Fivnat study of 3249 transfers

Fivnat, prepared by Royere D., Janny L, Clement P. and DeMouzon J.

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Introduction: Reports on the efficacy of frozen embryos transfers depending on the assisted reproduction technology procedure (IVF or ICSI) remain controversial. The aim of this retrospective study was to analyse frozen embryo transfer on a large scale, based on the data of the French national register.

Patients and methods: Study 1 was designed to analyse the practice of embryo freezing after IVF or ICSI from 1995 until 1999, among 142 552 cycles (89 centres). Study 2 was designed to evaluate the results of 3249 frozen embryo transfers from 1998 until 2000, including 34 centres. Results were analysed using univariate and multivariate analysis.

Results: Study 1: controlling for the study year and female age, we observed a lower total number of embryos, a higher number of embryos transferred and a lower number of embryos frozen after ICSI compared with IVF. However, the percentage of frozen embryos was similar in either procedure as a function of female age.

Study 2: the clinical pregnancy rate (CPR) after frozen embryo transfer was lower after ICSI compared with IVF (9.1 versus 11.6% and 11.2 versus 13.8% for CPR/thawing and CPR/transfer respectively). General data concerning the cycles which allowed embryo freezing revealed a lower female age, similar duration of ovarian stimulation, higher number of collected oocytes, lower number of embryos (8.5 ± 3.5 versus 9.2 ± 3.8), similar number of embryos transferred and same proportion of frozen embryos (48.7 versus 47.7%) after ICSI compared with IVF. Frozen embryo transfer differed neither by the number of embryos transferred (2.26 ± 0.78 versus 2.20 ± 0.75 for ICSI and IVF respectively) nor by their survival rate. In contrast, when the number and quality of the embryos transferred were kept similar, the chance of a successful pregnancy was lowered after ICSI compared with IVF (8.9 versus 15.9 % and 12.3 versus 19.1% for one and two embryos transferred with 100% survival respectively).

Conclusions: The lower efficacy of frozen embryo transfer after ICSI compared with IVF suggests a lower freezability, despite similarities in the usual criteria allowing such assessment. Whether these results may be explained by some inappropriate procedure of freezing embryos issued from ICSI or by some paternal effect on embryo development remains to be determined.

11.00–11.15

O-106. Array analyses and phenotyping of KO mice produced by ICSI

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Introduction: Chromosomal anomalies and malformations identified after ICSI are believed to be related to technical artefacts, parental karyotype and sperm maturity. Doubts on the safety of ICSI relate to the observations that following injection, sperm decondensation and pronuclear formation are delayed. This may cause problems in DNA synthesis and ultimately gene expression in the resultant embryos. The aim of the present study was to determine the expression profile and the phenotype of offspring produced by ICSI.

Materials and methods: $GPX^{-/-}$ KO mice lacking the gene coding for glutathione peroxidase were used for the experiments. These mice are hypersensitive to oxidative stress but show no overt phenotype. $GPX^{-/-}$ KO and wild-type oocytes were injected with a single spermatozoon using a Piezo micromanipulation system and the resultant embryos were transferred to foster females. For immunophenotyping, blood, spleen, thymus and bone marrow cell suspensions obtained from 6-week-old ICSI and naturally produced $GPX^{-/-}$ KO and wild-type offspring were analysed by FACS and system K100 haemanalyser. The cells from the different compartments were analysed for the expression of surface antigens and their colonization ability. Gene array filters were used to screen pan genes expression in the brain tissue of these offspring. The expression profiles of $GPX^{-/-}$ KO and wild-type ICSI offspring were identified and compared with those of $GPX^{-/-}$ KO and wild-type offspring from natural matings.

Results: Phenotyping analyses revealed that expression of some surface antigens on cells obtained from ICSI $GPX^{-/-}$ KO offspring was significantly different to that on cells obtained from $GPX^{-/-}$ KO offspring from natural matings. No differences in surface antigens were identified between cells from wild-type ICSI mice and wild-type mice produced from natural matings. Array analyses showed that gene expression profiles of ICSI $GPX^{-/-}$ KO and wild-type offspring were similar to those of offspring produced from natural matings.

Conclusions: The results demonstrate that ICSI does not alter gene expression. The differences in phenotype between ICSI offspring and offspring produced by natural matings were identified only in KO mice and may be a result of the embryos exposed to oxidative stress during culture. The differences may not affect longevity or reproductive abilities, but nevertheless require further investigation. This study was supported by the co-operative agreement HD 38228 from the NICHD, NIH.

11.15–11.30

O-107. Relativity of the concept 'poor responder' in assisted reproductive programmes

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Introduction: The aim of this study was to analyse the relativity of the poor responder concept according to the number of retrieved follicles after ovarian stimulation with recombinant FSH.

Material and methods: A total of 889 ICSI cycles between January 1998 and October 2001 were analysed. Of these, 153 couples with four or less retrieved follicles (179 cycles) were divided into two groups according to maternal age (group 1 ⩽35 and group 2 >35 years old) and compared with a control group (five to 16 follicles retrieved) with regard to mean gonadotrophin dose per patient (IU FSH), follicle and oocyte number, normal fertilization rates, number of embryos transferred per patient, and pregnancy and implantation rates.

Results: The results are shown in the table.

Variables	G1	Control G1	G2	Control G2
Cycles/patients	38/34	310/249	141/119	400/288
IU FSH	2754	2206	2954	2606
Mean maternal age ± SD	32.9 ± 1.6	31.2 ± 3.3	41.0 ± 3.5	39.1 ± 2.5
Follicles/oocytes (%)	112/72 (64.3)	3483/2381 (68.3)	402/252 (62.7)	3915/2564 (65.5)
Follicles/patient ratio	2.9	9.8	3.1	7.8
Normal fertilization (%)	75.9	80.9	77.2	83.9
Embryo transfer ± SD	1 ± 1.1	3.3 ± 1.7[a]	0.9 ± 0.8	2.8 ± 1.8[b]
Pregnancy rate/ patient (%)	20.6	28.5[a]	10.1	22.2[b]
Implantation rate (%)	21.6[a]	16.3	12.8	12.3

[a]$P < 0.05$ between group 1 and group 1 control.
[b]$P < 0.05$ between group 2 and group 2 control (Student's *t*-test).

Conclusions: Even though a lower number of follicles were recovered in the experimental groups, these patients can provide good quality oocytes and embryos, allowing embryo transfer with at least the same implantation potential. These results demonstrate the relativity of the concept 'poor responder' and indicate that the low retrieved follicle number after stimulation could be balanced by the good implantation rates. Indeed, the number of retrieved follicles alone is not a good parameter on which to select poor prognosis patients.

ART – Laboratory

Tuesday 2 July 2002
Hall E1

10.00–10.15

O-108. Recombinant human albumin as protein source in culture media used for IVF—a prospective randomized study

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Introduction: Different types of proteins have through the years been added to culture medias for IVF. Until the early 1990s, the most commonly used protein source in embryo culture was human serum. Today, human serum albumin (HSA) is the most frequently used protein source. However batch-to-batch variations exist and with the use of human-derived protein sources, i.e. HSA, the potential risks of transmitting viral or prion-carried diseases cannot be neglected. The first IVF culture medias with recombinant human albumin (rec-HA) are now a reality. Rec-HA is structurally identical to HSA, free from viral and prion contamination, has a greater homogeneity, lower endotoxin levels and no batch-to-batch