for several years at Bourn Hall Clinic. This study is carried out to investigate the effects of a soft catheter on the endometrium and endocervix. The data and visual images of the different types of trauma are presented.

Materials and methods: A prospective observational study was carried out on 34 women who were investigated during the luteal phase of a non-treatment cycle. A single operator performed a transvaginal ultrasonogram (TVERSUS), a mock embryo transfer (MET) and direct vision saline video hysteroscopy on all these women. The soft inner catheter was tried first and, if unsuccessful, the outer sheath, with or without the help of Allis forceps, was used. The use of a stylet was avoided.

Results: All women completed MET followed by hysteroscopy and three had MET but hysteroscopy was unsuccessful. The inner soft catheter alone was used in 25 (Group 1), an outer sheath was needed in six (Group 2), and Allis forceps in three (Group 3). In Group 1 ($n = 25$), evidence of trauma to the endometrium was noticed in six, to the endocervix in seven and to both in two and to neither in 10. In Group 2 ($n = 6$), evidence of trauma to the endometrium was noticed in two, to the endocervix in two, and to both in the remaining two. All in Group 3 ($n = 3$) showed trauma to the endocervix only. The three women who had MET but incomplete hysteroscopy showed visible trauma to the endocervix.

Table I. Effect of catheter in relation to the difficulty of embryo transfer

	Injury to endometrium only	Injury to endocervix/isthmus only	Injury to endometrium & endocervix	No injury
Group 1 ($n = 25$)	6	7	2	10
Group 2 ($n = 6$)	2	2 (+3)[a]	2	0
Group 3 ($n = 3$)	0	3 (+3)[b]	0	0

Group 1, catheter only; Group 2, catheter plus outer sheath; Group 3, catheter plus outer sheath plus Allis in two and catheter plus Allis in one.
[a]Three cases where hysteroscopy was not possible
[b]Three cases where MET was not possible

Table II. Nature of injury in relation to the site of trauma (and transfer catheter)

Endometrial trauma ($n = 8$)	Endocervical trauma ($n = 14$) (+3)[a]	Endometrial + endocervical trauma ($n = 4$)
Grooving × 1 (C)	Continuous bleeding × 2 (C+O; C+O+A)	Grooving endometrium + congestion Cx × 1 (C)
Perforation × 1 (C)	Bleeding + congestion × 4 (C × 2, C+O × 2, C+O+A × 1)	Grooving endometrium + bleeding Cx × 1(C+O)
SEH × 4 (C × 4)	Congestion only × 8 (C × 5, C+A, C+O × 2)	SEH + congestion Cx × 1(C)
Tear × 1 (C+O)	Congestion endometrium + congestion Cx × 1 (C)	Vascular congestion × 1 (C+O)

[a]Three cases where hysteroscopy was not possible.
C, catheter only; C+O, catheter + outer sheath; C+O+A, catheter + outer sheath + Allis; SEH, sub-endometrial haemorrhage; Cx, cervix.

Conclusions: Mechanical damage to the endometrium, and or endocervix, was evident in two-thirds of the cases following mock transfer with a soft embryo transfer catheter, and in all cases when an outer sheath and/or Allis forceps was used. Use of an outer sheath and/or Allis forceps was associated with bleeding from cervical mucosa but not from the endometrium. In all cases where blood was noted on the inside or outside of the catheter, the source was the endocervix. Further study is needed to investigate the clinical implications of these findings on the implantation process.

ART - laboratory: cryopreservation embryos

P-414. Success of human blastocyst vitrification by using minimum volume cooling methods

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Introduction: In some cases, a lack of blastocysts negates the transfer process. However, blastocyst transfer can be applied as a useful tool in human IVF programmes to decrease the risk of multiple gestation. Therefore blastocyst cryopreservation after transfer has become important to prevent wastage of supernumerary blastocysts. Recently, there have been some reports of a new vitrification method for domestic animals by using minimum volume cooling (MVC) methods. The aim of this study was to evaluate whether MVC method is useful for human blastocyst cryopreservation of clinical application.

Materials and methods: This study analysed 71 transfer cycles of blastocysts vitrified thawed between August 2000 and December 2002. After embryo transfer, supernumerary blastocysts were cryopreserved by MVC methods. Before vitrification, the blastocysts were first equilibrated for 10–15 min in 10% ethylene glycol (EG) and subsequently immersed in a vitrification solution containing 15% EG–15% dimethylesulphoxide (DMSO), 0.5M sucrose. Within 30 s a blastocyst was suspended on a 0.25 ml straw with a cut tip and plunged directly into filtrated liquid nitrogen. In order to prevent the blastocyst being exposed to contamination in liquid nitrogen, the 0.25 ml straw was loaded into a 0.5 ml straw and was stored in liquid nitrogen. The blastocyst was thawed in a 0.5M sucrose solution at 37°C and then the cryoprotectants were removed in 0.25, 0.1 and 0 M sucrose solution for 15 min in 30 µl droplets at 37°C. After removing the cryoprotectant, the blastocyst was cultured for 3 h until the transfer.

Results: A total of 128 blastocysts were thawed in 71 vitrified thawed cycles. One hundred and twenty-two blastocysts (95.3%) were kept alive and embryos could be transferred to all the patients. Thirty-five patients (49.3%) became pregnant and the implantation rate was 40.7%. When patients could be transferred good quality blastocysts, their clinical pregnancy rate was raised to 57.1% and the implantation rate was also raised to 48.9%. At present, 28 healthy babies were delivered by 20 patients.

Conclusions: The present data indicated that the MVC method was useful for human blastocyst cryopreservation of clinical application.

P-415. The outcome of thawed blastocysts originated from embryos of intermediate and poor quality

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Introduction: Experience with freezing/thawing of blastocysts (BL) originating from cycles with intermediate and poor quality embryos is scanty. Our aim was to evaluate the outcome of thawed blastocysts resulting from this group of embryos, which were unsuitable for freezing.

Materials and methods: From January 1999 to September 2002, two to four morphologically best classified embryos per cycle were transferred.

Supernumerary embryos with <20% fragmentation (grade I, II) were frozen. Embryos with >20% fragmentation (grade II–III, III, III–IV) were cultured further in G medium (Vitrolife, Sweden) to the BL stage. BLs were frozen after identification of a distinct inner cell mass (ICM), well laid down trophectoderm (TE) with sickle-type cells and thinning of zona pellucida (ZP). These BLs were classified as good, intermediate or poor. BLs were frozen on day 5 or 6, using a commercial kit (Irvine, CA). Thawing was by immersing the ampoule in water at room temperature. BLs were morphologically evaluated 2–4 h after thawing, according to the parameters: all cells have degenerated (Deg); the cells were clumped in a central mass away from the ZP (Shr- shrunken) partially expanded (Pexp) and fully expanded (Fexp) BL. Transfer was carried out on day 5 or after LH surge or on day 5 of progesterone administration.

Results: A total of 4075 intermediate to poor qualified embryos out of 992 treatment cycles were cultured to the BL stage. 455 BLs were frozen and 148 BLs in 68 treatment cycles were thawed. The Table I shows the distribution of the thawed BLs among the four morphological groups.

Table I

	BL morphology after thawing β			
	Deg (%)	Shr (%)	Pexp (%)	Fexp (%)
BL scoring at freezing				
Good	17/48 (35)	5/48 (10)	11/48 (23)	15/48 (32)[a]
Intermediate	14/40 (35)	12/40 (30)	12/40 (30)	2/40 (5)[a]
Poor	34/60 (57)	16/60 (27)	10/60 (16)	0/60 (0)
Total	65/148 (44)	33/148 (22)	32/148 (22)	17/148 (11)

[a]$P < 0.01$

Seventy-seven BLs (30 Shr; 30 Pexp and 17 Fexp) from 41 cycles were transferred, 1.8 ± 1.3 BLs per transfer. Seven of the pre-freezing good quality and one of intermediate pre-freezing quality BLs were implanted leading to six pregnancies (implantation and pregnancy rate of 10.4 and 14.6%, respectively). Four culminated in the delivery of four healthy babies and two are ongoing.

Conclusions: A tenth (11%) of intermediate and poor quality embryos, which would not have been suitable for freezing and discarded, will develop to good morphological BLs which can be frozen. BLs with good morphology before freezing have a significantly higher chance to re-expand after thawing and to establish a pregnancy.

P-416. Comparison of two vitrification protocol of human blastocysts using Cryoloop

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Introduction: The purpose of this study was to establish a reliable cryopreservation procedure for human blastocyst by rapid vitrification.

Materials and methods: For this experiment a total of 45 patients were divided into two groups: Protocol 1 ($n = 26$) and Protocol 2 ($n = 19$). All fertilized oocytes were cultured for 3 days, and then removed to a sequential medium until day 5 or day 6 for blastocyst formation. The vitrification and thawing of blastocysts was performed according to the vitrification technique involving ethylene glycol (EG), dimethyl sulfoxide (DMSO), Ficoll and sucrose as cryoprotectants for rapid vitrification using a cryoloop. Protocol 1: for vitrification, the blastocysts were equilibrated in 10% EG plus 10% DMSO for 2 min at room temperature, and then placed into vitrification solution of 20% EG, 20% DMSO and 10 mg/ml Ficoll70 with 0.65 M sucrose. Finally, the blastocysts were plunged immediately into liquid nitrogen (LN2) for approximately 20 s. For the thawing procedure, the blastocysts were immediately placed into 0.5 M sucrose solution for 2 min, and sequentially paced into 0.25 M for 3 min, and then removed into 0 M sucrose for 5 min at 37°C. The thawed blastocysts were transferred to patients with hormone replacement treatment. Protocol 2: blastocysts were equilibrated in 7.5% EG and 7.5% DMSO for 2 min, and removed into 15% DMSO, 15% EG, 10 mg/ml Ficoll70 and 0.65 M sucrose for approximately 20 s at 37°C. For the thawing procedure, the blastocysts were immediately placed into 0.25 M sucrose in solution for 2 min, and sequentially paced into 0.125 M for 3 min, and then removed into 0 M sucrose for 5 min at 37°C. Other techniques were almost the same as Protocol 1.

Results: The two experiment groups were comprised of women of similar ages (34.3 ± 4.1 versus 35.6 ± 4.7). Survival and re-expansion rates were similar in both groups (83.1% versus 76.5%), showing no significant difference. The average number of blastocysts transferred were similar (2.4 ± 1.1 versus 2.2 ± 0.8), showing no significant difference. The implantation rate in Protocol 1 was higher than in Protocol 2 (22.4% versus 10.3%). Also, the pregnancy rate per transfer in Protocol 1 was higher than in Protocol 2 (50.0% versus 22.2%, $P < 0.05$).

Conclusions: These results suggest that Protocol 1 is a useful method of rapid vitrification for blastocysts improving both their implantation and pregnancy rates.

P-417. Single embryo transfer in frozen embryo transfer cycles: results from 1998 to 2002

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Introduction: Our elective single embryo transfer (SET) programme in fresh cycles have reduced the multiple pregnancy and delivery rates from 25% to 7.5% and from 25% to 5%. When SET is combined with freezing of extra embryos, the cumulative delivery rate per oocyte retrieval is high. As frozen embryo transfers (FETs) also result in multiple pregnancies, we have started to transfer one embryo also in frozen cycles.

Materials and methods: During 1998 and 2002, 1368 FETs were performed. The embryos were frozen and thawed using the standard PROH method. Embryos were transferred if at least 50% of the blastomeres survived, fragmentation was <50% and there was no multinuclearity. Two embryos were transferred in 779 (57%) cycles and one embryo in 589 (43%) cycles. Embryo transfer was performed either during a spontaneous (844 FETs) or during a hormonally substituted cycle (524 FETs).

Results: The overall clinical pregnancy rate (PR) was 29.5% (403/1368; range 20.3–34.7%). In spontaneous cycles, the PR was 28.9% (244/844) and in substituted cycles 30.3% (159/524). In 1998, 78 out of 276 FETs (28%) were SETs, most because only one embryo survived. In 15 cycles, SET was planned either due to patients' own wish or medical reasons to avoid twins. In 2001, 304 (52%) out of 586 FETs were SETs. Of these 170 were planned to be SETs, mostly due to the patients' own wish. The PR in SETs in 2001–2002 was 28.3% (86/304) and in the planned SET cycles it was 31.8% (54/170). Elective SET was performed in 73 cases, because more than one embryo survived and PR was 37.0% (27/73). At the same time, the PR in two-embryo transfer cycles was 40.4%. The multiple pregnancy rate in FET cycles decreased from 18% to 5.1%.

Conclusions: SET policy can be adopted to cryopreservation programmes. Good pregnancy rates with low multiple delivery rates can be maintained when individualised freezing and thawing practices are applied. The transfers can be performed either during spontaneous or substitution cycles, depending on patient characteristics.

P-418. High survival rate of human blastocysts after preimplantation genetic diagnosis and vitrification

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Introduction: Preimplantational genetic diagnosis (PGD) allows subfertility due to genetic base to be overcome. The full offering of this technology requires efficient blastocyst cryopreservation to store surplus embryos and to keep open future reproductive options. Among the

techniques available to cryopreserve PGD-derived biopsied blastocysts, the slow-freezing has rendered the lowest survival results. In the present study, we report an in-vitro assay on vitrification of PGD-derived blastocysts and our first clinical trial.

Materials and methods: In the in-vitro assay, we included 19 chromosomally abnormal blastocysts from five patients, at different developmental stages: two at early (EB), four cavitated (CB), two expanded (EB), nine initiating hatching (HiB) and two hatched (HB) blastocysts. For vitrification, embryos were incubated in 20% EG for 4 minutes, removed to 25% ethylene glycol and dimethylsulphoxide in phosphate-buffered saline supplemented with serum for 45 s. Embryos were then loaded into plastic straws, identified, sealed and plunged into liquid nitrogen. For warming, straws were immersed into a 37°C water bath for 5 s. Dilution of cryoprotectants was performed by six-step incubation in decreased sucrose solutions and finally cultured in CCM (Vitrolife) at 37°C in a 5% CO and 95% relative humidity. Following 6–12 h of culture, embryo survival was assessed by the morphological integrity and blastocoele re-expansion. For the clinical trial, we included 12 women out of our PGD programme that had surplus normal blastocysts to vitrify. After fresh blastocysts transfer, only two patients asked for their vitrified embryos. After warming, embryos were cultured for 6–12 h in the above-referred conditions and only those with healthy morphological appearance were transferred. On day 9 and 16 post-transfer, patients were submitted to pregnancy tests (HCG blood levels) and ecography.

Results: After warming, 73.7% of vitrified abnormal blastocysts survived and progressed in their in-vitro development. Differential survival rates were observed according to the blastocyst developmental stage (EB: 2/2, CB:2 /4, EB: ½, HiB: 9/9 and HB: 0/2). In the clinical trial, three embryos were involved (two HiB and one CB). Only one HiB survived, was transferred and resulted in an ongoing pregnancy.

Conclusions: These results indicate that vitrification of PGD-derived biopsied blastocysts could be included in our technological background, allowing PGD to be a complete programme.

P-419. Vitrification of blastocysts after artificial hatching on day 4: an approach to cryopreserve biopsied embryos

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Introduction: Opening of the zona pellucida of biopsied human embryos is held responsible for the high rate of embryos lysis post-thawing. Therefore, in order to cryopreserve embryos post-PGD, there is a need to adopt a strategy. One approach may consist of extending the culture of the biopsed embryos to the blastocysts stage and to cryopreserve them. In a first step, we have to analyse the biopsed embryos, if hatching blastocysts survive the cryopreservation procedure. We present our preliminary results on the freezing ability of hatching blastocysts with artificial open zona pellucida, which were vitrified using the hemi-straw technique.

Materials and methods: Three-dimensional partial zona dissection was performed on day 4 on 19 compacted morulae. Following a culture period of 24 h in CCM medium, 14 hatching or totally hatched blastocysts were vitrified using the hemi-straw procedure. Before vitrification, hatching blastocysts were equilibrated in two steps in mixtures of ethylene glycol and dimethylsulphoxide of 20% and 40%, respectively. An approximate volume of 0.3 µl of cryoprotectant solution containing the blastocysts were deposited to the tip of a hemi-straw and then instantly plunged into LN2. For warming, the hemi-straw was immersed in 0.5 M sucrose before dilution into culture medium.

Results: A total of 14 blastocysts from five patients with artificially open zona pellucida were vitrified. Eleven (79%) embryos survived the vitrification procedure and underwent a culture period of 24 h. A total of 11 blastocysts were transferred in five patients and four pregnancies (two already delivered) were obtained (80%). The implantation rates per vitrified and transferred embryos were 29% and 36%, respectively.

Conclusions: Vitrification of hatching blastocysts using the hemi-straw technique seems to have no adverse effect on their developmental potential. Culture of embryos post-biopsy to the blastocysts stage and their cryopreservation using the vitrification protocol with the hemi-straw is a good alternative to the slow freezing of early cleavage stages.

ART - laboratory: cryopreservation gametes

P-420. Minimal sperm parameters for effective sperm freezing in patients with severe male factor infertility candidates for ICSI

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Introduction: This study aimed to find out the minimal sperm parameters needed for effective sperm freezing in patients with severe oligoasthenozoospermia with a sperm count <0.1 million/ml, and testicular spermatozoa retrieved from non-obstructive azoospermic patients candidate for intracytoplasmic sperm injection.

Materials and methods: This study was conducted at the Adam International Clinic and included 110 patients who underwent cryopreservation trial for ejaculated (51 samples) and testicular spermatozoa (59 samples) and 43 ICSI cycles.

Results: The samples were classified into two categories: (i) injectable, where the post-thawed total motile sperm were sufficient to inject all available oocytes; and (ii) non-injectable, where post-thawed total motile sperm were insufficient to inject all available oocytes. In 43 cases, ICSI was done using frozen thawed spermatozoa. For the other 76 cases, fresh ejaculated or testicular spermatozoa were used for ICSI due to better fresh sperm quality or poor post-thawing testicular sperm parameters. The minimal threshold of total sperm count and total motile sperm count before cryopreservation, as determined by the receiver operating characteristic (ROC) curve analysis, necessary for recovery of viable spermatozoa suitable for injection of the all available oocytes, was found to be 194 sperm and 41 sperm, respectively. The fertilization, clinical pregnancy, and implantation rates were 275/496, 18/43, and 28/179 representing 55.4, 41.9, 15.6%, respectively. These results are comparable to our results using fresh ejaculated or testicular spermatozoa.

Conclusions: To ensure recovery of an adequate number of spermatozoa after thawing, the pre-freezing total sperm count must not be less than 194 sperm and total motile sperm count must not be less than 41 sperm. Patients with severe oligoasthenozoospermia undergoing sperm freezing can be counselled whenever motile spermatozoa are recovered after thawing. ICSI outcome is favourable in samples with higher sperm count.

P-421. Cryopet: cryopreservation of minute volumes of testicular sperm suspensions

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Introduction: Using modern techniques for the retrieval of testicular sperm, only a minute volume of tissue needs to be aspirated or excised. Often the number of motile sperm in this tiny amount of tissue is sufficient for several ICSI treatment cycles. Considering the risk, however

small, of impairment of testicular function associated with surgical sperm retrieval, it is desirable to minimize the number of such interventions and maximize the utility of each episode. Conventional sperm freezing techniques use 2503.25 l or 500.25 l straws, which make them less suitable for cryopreservation of small amounts of this precious material. This study describes a technique for freezing small volumes of testicular sperm using the Cryopet technique.

Materials and methods: Testicular sperm extraction (TESA) material was moved to sperm freezing medium (Sage Biopharma, Bedminster, NJ, USA) using a 600 µm diameter Flexipet and a Flexipet flushing device (Cook IVF, Eight Miles Plain, Australia), and taken up in a 10–15 mm column (volume 3–4 µl). The Flexipets were put into 0.25 ml embryo freezing straws, which were then heat sealed on each side of the Flexipet. The straws were frozen in a liquid nitrogen vapour gradient. The straws were thawed at room temperature and the Flexipets were emptied into centrifuge tubes containing about 100 µl of HEPES buffered sperm wash medium (Sage Biopharma). This was centrifuged at 300 *g* for 10 min and the supernatant discarded. The micropellet was resuspended in about 25 µl of medium and 1–2 µl aliquots were then dispensed in droplets in a Petri dish. The dish was then examined for the presence of viable sperm and these were then used for ICSI.

Results: Of 58 Flexipets frozen with testicular sperm from six patients, five have been thawed, three of which were successfully used for ICSI in two patients (14/15 fertilized, 12/14 cleaved; 3/4 fertilized, 3/3 cleaved). All thawed samples had motile sperm that were easily recovered and usable for ICSI. The longevity in room temperature of the frozen/thawed spermatozoa was good (2–8 days).

Conclusion The Cryopet method is efficacious for cryopreservation of small volumes of testicular spermatozoa.

P-422. Limited recovery of meiotic spindles of metaphase II oocytes after vitrification and thawing

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Introduction: The integrity of meiotic spindles is crucial during cryopreservation of metaphase II (MII) oocytes. The thawed oocytes have been cultured for some time before further manipulation. In this study, the spindle status of oocytes after vitrification were assessed using Polscope at fixed interval. The Polscope images were further correlated with immunofluorescent staining.

Materials and methods: Mature MII oocytes were collected from ICR mice superovulated by pregnant mare's serum gonadotrophin and HCG injections. Only oocytes with good spindle morphology by Polscope subsequently underwent vitrification thawing protocol. The oocytes were vitrified with 5.5 M ethylene glycol and 1 M sucrose-containing Dulbecco phosphate buffered saline. They were loaded into thin-walled pulled straw and were stored in liquid nitrogen until thawing. After thawing, the oocytes were evaluated by Polscope every 15 min for 3 h. The oocytes were fixed when there was no change of spindle imaging for three consecutive assessments or at the end of 3 h observation. The fixed oocytes were then evaluated with immunofluorescent staining for tubulin (fluorescein isothiocyanate conjugated antibody) and chromatin (Hoechst 33258). Thawed oocytes that remained intact during observation and before fixation were included for analysis.

Results: In total, 150 intact thawed oocytes were included for analysis. Most of the spindle images appeared during the first hour of observation. At the end of 3 h observation, 42 (28%) oocytes did not have spindle images. After immunofluorescent staining, nearly all 42 oocytes were absent of spindle images or with disrupted spindle morphology. Among those 108 oocytes with spindles images detected by Polscope, 66 had good spindle images while 25 had signs of activation. The spindle images by Polscope were well correlated with those by immunofluorescent staining.

Conclusions: Meiotic spindles have poor recovering ability after thawing with present cryopreservation protocols. In the present protocol, a relative high percentage of oocytes was activated during cryopreservation. Polscope could be used as a tool of assessment for meiotic spindle in cryopreservation to improve the cryopreservation procedure.

P-423. Evaluation of the protective effect of L-carnitine and acetyl-L-carnitine on human spermatozoa during freezing and thawing

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Introduction: L-carnitine (LC) and acetyl-L-carnitine (ALC) are known to contribute to the antioxidant defences of the spermatozoa. The aim of this study was to assess the protective effect of carnitine derivatives on oxidative damage to spermatozoa during cryopreservation and thawing.

Materials and methods: Semen samples were collected from 20 normal healthy volunteers. Specimens were divided into two aliquots (A and B) and each diluted in Ham's F-10 medium to a uniform concentration of 20×10^6/ml. Aliquots A, were further divided and into each L-carnitine or acetyl-L-carnitine were added in concentrations of 0, 10 and 30.25 M separately. All aliquots were cryopreserved for 3 days. Sperm motility, viability, fertilizing capacity, reactive oxygen species (ROS) formation and the level of lipid peroxidation (LPO) were analysed before and after cryopreservation.

Results: The sperm viability showed significant higher levels in aliquots A, which was directly correlated with the concentration of ALC ($P < 0.05$). Also, the sperm fertilizing capacity was significantly higher, and ROS generation and LPO were significantly lower in proportion to the concentration of LC and ALC ($P < 0.05$ for each). There was no improvement in sperm motility with the increase in concentration of acetyl-L-carnitine ($P > 0.05$).

Conclusions: Our results suggest that carnitine derivatives can effectively scavenge free radicals in semen specimens. Therefore, supplementation of carnitine to the cryoprotectant can prevent oxidative damage incurred by spermatozoa during freezing and thawing.

ART - laboratory: cryopreservation gonads

P-424. Oocyte collection during ovarian cortex cryopreservation: a combined approach

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Introduction: Young single female patients are counselled to preserve fertility prior to chemotherapy for malignancy or bone marrow transplantion. Since IVF requires a male passisted reproduction treatmentner and delays chemotherapy by several weeks, it is not usually an option for these patients. Ovarian cortex cryopreservation is commonly performed in many hospitals as it offers the potential of freezing many small follicles on an urgent basis. However, this procedure has not yet resulted in a human conception. Moreover, cooling damage and transplantation-induced ischaemia result in the loss of all large follicles. Since ovarian tissue contains oocytes that will inevitably be lost, it is only logical to retrieve and freeze them separately. Such an approach could utilize the ovarian tissue for obtaining both oocytes and ovarian cortex.

Materials and methods: Laparoscopic unilateral oophorectomy was performed in nine patients for ovarian cortex cryopreservation. The ovaries were immediately transferred to the laboratory in Leibovitz medium on ice. Cumulus-oocyte-complexes (COC) and the oocytes that emerged from the follicles were collected with micropipettes and denuded of cumulus cells. Oocytes were transferred to P1 medium containing 10% synthetic serum. The ovarian cortex was sliced to thin 1 mm^3 fragments, which were cryopreserved. In-vitro maturation (IVM) and ICSI were performed in patients requesting fertilization.

Results: Search for oocytes was performed in nine patients admitted for ovarian cryopreservation. The mean age of patients was 22.3 ± 6 years. Patients were treated for advanced osteosarcoma ($n = 2$), acute myelocytic leukemia (AML) ($n = 2$), bone marrow transplantation (BMT) for thalassemia major ($n = 2$), Hodgkin's lymphoma ($n = 1$), non-Hodgkin's lymphoma (NHL) ($n = 1$) and acute vasculitis (Sussak's syndrome) ($n = 1$). From seven of these nine patients (78%) a total of 32 oocytes (range 0–7) were found in either the follicular aspirate or in the collecting medium.

Conclusions: Since a few antral follicles can be found in the ovary at any stage of the cycle, we recommend rescuing oocytes by aspirating all antral follicles from ovaries prior to ovarian cortex cryopreservation. Immature oocytes aspirated from unstimulated ovaries removed for fertility preservation could be cryopreserved or in-vitro matured. ICSI of these oocytes yields embryos which could subsequently be cryopreserved. This approach not only rescues these oocytes but also expands the range of cryopreservation possibilities, thus increasing the potential for urgent fertility preservation in patients at risk of ovarian failure.

P-425. Oocyte maturation in xenografted cryopreserved human ovarian tissue

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Introduction: In a previous study we demonstrated that antral follicles could develop in human frozen/thawed ovarian tissue following xenografting1. Subsequent studies have also demonstrated that antral follicle development is reproducible and that follicular components are capable of undergoing periovulatory changes. The present study reports the observation of mature oocytes within follicles undergoing periovulatory changes following administration of HCG.

Materials and methods: Ovarian tissue was donated from three patients at risk of loss of fertility. Thin slices of ovarian cortex were cryopreserved and the cryopreserved tissue was grafted under the kidney capsule in SCID mice. Injections of gonadotrophin (1 U/ml recombinant FSH) commenced on day 7 and continued every second day to the completion of the study (26 weeks). An i.p. injection of 20 IU HCG was given 30–36 h prior to the completion of the study, after which the kidneys were removed, examined for follicular development and fixed for histology.

Results: Tissue from the three patients was xenografted into a total of 12 mice (24 sites). Antral follicles were present on the majority of sites (67%, 16/24), over half of which (on histological examination) had more than one antral cavity on the site (9/16). Histological evidence of a response to HCG was observed in all (32) antral follicles ranging from disorganization and degradation of granulosa and theca cell layers to rupture of the follicle wall. Concurrent within these follicles was the mucification of the cumulus complex. In 69% (22/32) of these follicles, evidence of a response to HCG (resumption of meiosis) was also observed within the oocyte. Oocytes that appeared not to have responded to the HCG trigger were within small follicles (<2 mm in diameter). Germinal vesicle breakdown was observed in 12 oocytes, progression to metaphase I observed in five oocytes and metaphase II in a further five oocytes.

Conclusions: The study has demonstrated that follicles, cryopreserved at the primordial stage within human ovarian tissue, are capable of development to the periovulatory stage following xenografting. Concomitant with these HCG-induced periovulatory changes within the somatic cells of the antral follicle are maturation events within the gamete, resulting in the presence of metaphase II oocytes.

P-426. Effect of cryopreservation on the hsp90 expression in mouse ovary

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Introduction: The heat shock protein (hsp) family is related to protective mechanism of cells by environmental changes. This study was performed to evaluate the effect of cryopreservation on hsp90 expression in mouse ovarian tissue.

Materials and methods: Cryopreservation of mouse ovarian tissue was carried out by slow freezing method. The mRNA level of hsp90 expression in both fresh and cryopreserved mouse ovarian tissue was analysed by RT-PCR. The protein expression of hsp90 was evaluated by Western blot analysis and immunohistochemistry.

Results: The mRNA and protein of hsp90 were expressed in both fresh and cryopreserved mouse ovarian tissue. The amount of hsp90 mRNA was increased in cryopreserved ovarian tissue after 30 min and 1 h after thawing and incubation. The amount of hsp90 protein was increased in the cryopreserved ovarian tissue after 6 h of the incubation in Western blot analysis. In immunohistochemical study, hsp90 protein was localized in cytoplasm of oocytes and granulosa cells. A significant level of immunoreactive hsp90 protein was detected in theca cells in contrast to the weak expression in ovarian epithelial cells.

Conclusions: These results showed an increase of hsp90 expression in both mRNA and protein level in the cryopreserved mouse ovarian tissue. It can be suggested that hsp90 may play a role in the protective or recovery mechanism against the cell damage during cryopreservaion.

ART - laboratory: embryo selection

P-427. Early cleavage and further embryonic development

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Introduction: The choice of embryos to be transferred is usually based on day 2 embryo morphology. However, it has been demonstrated that early cleavage observed 26 h post-ICSI procedure is a good predictor of pregnancy as well. The aim of this work was to study the influence of early cleavage on further embryonic development.

Materials and methods: A total of 1627 zygotes obtained after 303 ICSI procedures were observed 26 h post-injection and classified using the following criteria: (i) 2 pronuclei (2PN) continuing stage, (ii) 2 pronuclei breakdown stage (2PNBD) corresponding to meiotic spindle formation, and (iii) first embryo division stage (early cleavage) described using the following classification. (A) Two regular blastomeres without any fragmentation, (B) two blastomeres of unequal size or shape without any fragmentation, and (C) type A and B embryos with fragmentations and other embryos. Forty-eight hours after oocyte retrieval, embryos were classified using an embryo score (1–4) in which score 3 and score 4 embryos are embryos with the highest implantation rate (good embryos). Statistical analysis was done using χ^2 test.

Results: The percentage of score 3 plus score 4 embryos at transfer time was higher in the early cleavage (EC) group than in the no early cleavage (NEC) group (59.1% versus 34.4%; $P<0.001$). Regarding the early cleavage group, embryos displaying two regular blastomeres (group A) gave rise to more good embryos (78.5%) than embryos with two irregular blastomeres (group B: 53.1%; $P<0.001$) and than embryos with fragmentations (group C: 28.5%; $P<0.001$). The results of the study are summarized in Table I.

Table I

	Early cleavage				No early cleavage		
	A	B	C	Total EC	2PN	2PNBD	Total NEC
Stage 26 h							
Nb	415	128	239	782	435	410	845
Score 3+4 embryos at 48 h							
Nb	326	68	68	462	123	168	291
%	78.5	53.1	28.5	59.1	28.3	40.9	34.4

Conclusions: This study demonstrates that day 2 embryo morphology is strongly correlated to early cleavage observed 26 h post-ICSI. Moreover, the classification of early embryos defined in this work allows us to predict the development of embryos having the best implantation prognostic. In further work, we will assess the efficacy of a new transfer policy based on the combination of both the 26 h embryo classification and the 48 h embryo score.

P-428. Impact of the number of 8-cell embryos at transfer with respect to the German Embryo Protection Law

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Introduction: The German Embryo Protection Law prohibits the culture of more than three pronuclear stage (PN) embryos and the cryopreservation of cleavage stage embryos. This means that this process does not allow embryo selection on the day of transfer. This retrospective analysis of IVF and ICSI cycles compares the outcomes of day 3 transfers (D3ET) with different morphological embryos from 1 January 2001 to 31 December 2002.

Materials and methods: A total of 172 IVF and 292 ICSI cycles were stratified by the number of 8-cell embryos available for D3ET (two, one or none were transferred). The average maternal age was 34 years (IVF) and 33 years (ICSI). The average number of embryos transferred was 2.05. Statistical analysis was carried out by means of the χ^2 test.

Results: In 70% of IVF cycles ($n=121$) and 52% ICSI cycles ($n=151$) at least one 8-cell embryo was transferred. Furthermore, two 8-cell embryos were available in 41% (71/172) of IVF cycles but only in 23% (67/292) of ICSI cycles. With two 8-cell embryos for D3ET, the rate of viable pregnancy was 46% and 36% (IVF versus ICSI) ($P=0.002$). This rate decreased to 30% when only one 8-cell embryo was transferred (IVF and ICSI). In addition, no 8-cell embryo was available in 30% of IVF cycles ($n=51$) and 48% of ICSI cycles ($n=141$). However, with no 8-cell embryo, only 14% and 11% pregnancies resulted from IVF versus ICSI ($P=0.29$).

Conclusions: The data from this study show that the rate of pregnancy depends on the number of 8-cell embryos per transfer available on day 3. In addition, these data have shown clearly the disadvantage of the German Embryo Protection Law, because it leads to a high number of cycles (41%; 192/464) with a lower likelihood of pregnancy (~12%). It is concluded that embryo selection at the time of embryo transfer is highly beneficial for the patients, because the number of repeated cycles may be reduced, and routine pregnancy rates may be increased in this way.

P-429. How to improve embryo selection

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Introduction: The relatively low initial success rate following in-vitro procedures indicates that the criterion of selecting embryos, based on their morphology and the cleavage rate, is not sufficient. In this study we checked the ability of a modified pronuclear scoring system to assess the quality of zygotes and early embryos following ICSI.

Materials and methods: The present prospective study juxtaposed two patient groups treated with ICSI for infertility problems: The first one included 97 couples treated from January to December 2001. In this group the selection of the embryos was based on the number of blastomers and the fragmentation. The second group included 108 couples treated from January to December 2002, in whom the new scoring system was applied. The pronuclear scoring performed was based mainly on the recently published system of Scott and Smith. The alignment of nucleoli at the junction of the two pronuclei and the position of the pronuclei were evaluated. To emphasize the importance of the synchrony between the two pronuclei, the nucleoli of polarized form were given a score of 20. Nucleoli starting to align were scored 10 and all the rest of the patterns were scored 0. Zygotes were reexamined 25–28 h after ICSI and pronuclear membrane breakdown was scored 10 and early cleavage was scored 20.

Results: Both the implantation rate and the pregnancy rate increased significantly with the number of full score zygotes and early cleavage embryos in transferred embryos. The use of the new scoring system led to 56% biochemical and 48% clinical pregnancy rate, which was significantly higher ($P<0.05$) than that in the first group where selection of the embryos for transfer was performed on the basis of number of cells and the extent of fragmentation: 31% biochemical and 23.8% clinical pregnancy rate, respectively.

Conclusions: The combination of pronuclear scoring and early cleavage acts as a double-check test to select embryos for embryo transfer. It proved to be a useful prognostic parameter that may optimize embryo selection further.

P-430. Combined score system of pronuclear morphology, early cleavage, and embryo development is highly predictable of pregnancy outcome in human assisted reproduction treatment programmes

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Introduction: Zygote morphology and the time of cleavage are related to embryo quality and successful pregnancy. In this study, we examined the relationship between combined score for pronuclear (PN) morphology, early cleavage, embryo development, and further clinical pregnancy outcome.

Materials and methods: A total of 229 patients who had undergone IVF-ET from May 2002 to November 2002 at Mizmedi Hospital were studied. PN morphology was graded according to nucleoli pattern ($n=994$) and early cleavage (EC) was evaluated at 24–26 h post-insemination or ICSI ($n=667$). Cleaved embryos were transferred to patients at day 3 after embryo evaluation. The group of embryo transfer was divided into five groups according to the nucleoli pattern of PN and EC: group A, only P1 + P2 embryo transfer; group B, all pattern except P1 + P2 embryo transfer; group C, only EC embryo transfer; group D, no EC embryo transfer; group E, combined group A and group C.

Results: In nucleoli pattern of PN, a significantly higher rate of good quality embryos were derived from pattern 1 and 2 (same pattern of several nucleoli present in both PN, P1/P2) zygotes than P6 zygotes. Also, developments of the EC group were significantly more rapid than that of the 2PN group at the same time. Moreover, P5/P6 embryos were late to start cleavage than P1/P2 embryos. The pregnancy rate of group A was

higher than that of group B (35.8 versus 16.0 %). Also, the clinical pregnancy rate of group C was higher compared with group D (36.4 versus 23.6%). Furthermore, the clinical pregnancy rate of group E was higher than that of groups A or C (44.1 versus 35.8 and 36.4 %, respectively).

Conclusions: Embryo development and clinical pregnancy rate were related to nucleoli pattern of PN and the time of cleavage start. Taken together, we suggest that although PN morphology and EC are important factors of predictive value in the assessment of human embryo quality, a combined evaluation of PN morphology and EC of zygotes may be a more obvious indicator for the outcome of pregnancy in human assisted reproduction treatment programmes.

P-431. Early embryo development is an indicator of implantation potential

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Introduction: One of the major challenges in clinical assisted reproduction treatment has been the ability to select accurately those embryos most likely to implant and give rise to a clinical pregnancy. Currently, there are a number of methods for selecting embryos for transfer, including developmental stage, morphology, and more recently, pronuclear morphology and early cleavage. The aim of this study was to assess the prognostic value of early cleavage at 25–27 h post-insemination, and to include syngamy as a further category at this assessment.

Materials and methods: The study included 348 couples who underwent 352 cycles of IVF/ICSI between August 1999 and September 2001. Ovarian stimulation was achieved using standard protocols. A total of 2450 normally fertilized zygotes were cultured in proprietary media for 2 days before embryo transfer. All normally fertilized zygotes were checked within 25–27 h of insemination for stage of development, and classified as A (pronuclear), B (syngamy) or C (2-cell). Only cycles that led to embryo transfer with embryos of equivalent classification at 25–27 h were included in analysis.

Results: Of the 2447 embryos scored, 967 (39.5%) were at the pronuclear stage, 757 (30.9%) were at syngamy, 615 (25.1%) were at the 2-cell stage and 108 (4.4%) did not fit any of the three categories when scored. Implantation rates were significantly higher if the embryos transferred were all group B or C (22.4% and 32.1%, respectively) compared with group A (10.3%) ($P < 0.001$). There was no significant difference between the groups in maternal age, basal serum FSH concentration, number of eggs collected or number of embryos available for transfer.

Conclusions: This data further supports evidence that early cleavage can be an independent predictor of pregnancy. Embryos at syngamy 25–27 h after insemination showed a significantly higher implantation rate compared with pronucleate zygotes ($P < 0.05$) and should be included in the observations. Categorization of embryos in this way represents a straightforward and non-subjective method of assessing embryo quality at an early stage of development and identification of embryos in syngamy may be of particular use in patients where early cleavage is not evident.

P-432. Chromosomal abnormalities and morphology on day 3 embryos

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Introduction: Embryo development and blastocyst rates have been correlated with chromosomal abnormalities. The aim of this study was to assess the relationship of blastocyst rates and chromosomal abnormalities with embryo quality on day 3, in terms of cell number and fragmentation rate (FR).

Materials and methods: This was a retrospective study of 490 embryos, from 83 patients, of our preimplantation genetics diagnosis (PGD) programme (January 1999 to December 2002). Inclusion criteria were patients ⩽37 years of age with analysis of seven chromosomes (13, 16, 18, 21, 22, X and Y) by fluorescence in-situ hybridization (FISH). Patients were treated for: recurrent miscarriage ($n = 42$), implantation failure ($n = 20$), abnormal FISH results in sperm ($n = 7$) and aneuploidy screening ($n = 14$). ICSI was performed in all the cycles. On day 3, cell number and FR were checked and normally developing embryos were biopsed and analysed. Embryos were classified into five groups according to cell number: <6, 6, 7, 8 and >8 cells and into two groups according to FR: ⩽20 % and >20%. Statistical comparisons between groups were established by the χ^2-test.

Results: Blastocyst rates were significantly ($P < 0.001$) higher in embryos with ⩽20% FR (42.7%) compared with embryos with >20% FR (15.5%). There was also an increased incidence of chromosomal abnormalities in embryos with >20 % FR (77.8%) compared with embryos with ⩽20% FR (65.6%), without statistical significance. When embryos had ⩽20% FR, blastocyst rates were significantly lower ($P < 0.001$) if the number of cells was ⩽6 compared with all the other embryos (14.5% versus 55.4%). Regarding chromosomal abnormalities, 8-cell embryos had significantly lower incidence (57.0%) compared with ⩽6-cell embryos (75.4%) and >8-cell embryos (72.3%), as well as 7-cell embryos compared with <6-cells (61.0% versus 77.8%) ($P < 0.05$). In embryos with >20% FR, chromosomal abnormalities and blastocyst rates were not significantly different according to the cell number on day 3.

Conclusions: Blastocyst rates and chromosomal abnormalities in embryos with ⩽20% FR are strongly correlated with cell number on day 3. However, high FR on day 3 embryos showed a detrimental effect in the incidence of chromosomal abnormalities and blastocyst rates, independently of the cell number.

P-433. Looking for the highest implantation rate in IVF: a four-step sequential evaluation of the embryos

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Introduction: Selection of embryos with the highest implantation potential is a critical point for the challenge of single embryo transfer. We describe a four-step sequential evaluation (SE) of embryo development and weight the relative relevance of each step with the help of transfers ending with 100% implantation.

Materials and methods: Embryos were cultured in sequential media until day 3 and scored at four key moments: I, pronuclear stage (18–20 hpi-hours post-insemination); II, early cleavage (25–26 hpi); III, day 2 (42–44 hpi); and IV, day 3 (66–68 hpi). High implantation prognosis at each step was defined by the following criteria: (I) two opposed and equally-sized pronuclei containing nucleoli with similar distribution, size and number; (II) 2-cell stage or syngamy; (III and IV) ⩾4-cell and ⩾7-cell embryos showing no more than one minor defect, i.e. <20% fragmentation or slightly uneven blastomeres or slightly granular cytoplasm. IVF cycles were divided into two groups. In the SE- group (38 cycles), embryos were scored on step IV as the unique criterion. In the SE+ group (66 cycles), embryos have been submitted to the four-step SE. In this case, transferred embryos were those combining several high prognosis criteria, according to the following priority order: IV, II, III, I.

Results: The SE- and SE+ groups did not differ in maternal age, number of retrieved oocytes, number and mean quality of replaced embryos. Pregnancy and implantation rates (IR) in the SE- group (12/38, 31.5% and 17/90, 18.9%) and the SE+ group (24/66, 36.4% and 35/144, 24.3%) were not significantly different. In transfers ending with 100% implantation in the SE+ group, eight of the 23 embryos (35%) encompassed the four quality criteria, while nine (39%), two (9%) and four embryos (17%) fulfilled 3, 2 or 1, respectively, of those criteria. The most relevant

criterion was early cleavage (II), demonstrated by 22/23 embryos (91%). Criteria I, III and IV were seen in 16, 16 and 15 embryos, respectively. In cycles where the quality of implanted embryos was known, IR of poor prognosis day 3 embryos was four-fold higher in the SE+ group than in the SE- group (13/72, 18% versus 2/43, 4.6%; not significant), while IR of high prognosis embryos was the same in the SE+ and SE- groups (18/63, 28.6% versus 10/36, 27.7%). In this study, 29.8% of cycles had no high prognosis embryo available on day 3.

Conclusion: Sequential evaluation of embryos does not increase significantly pregnancy or implantation rates but has a major importance to select embryos if no high prognosis embryo is available on the day of transfer. Among all parameters evaluated, early cleavage is the strongest predictive criterion of implantation. Sequential evaluation may be greatly helpful in case of single embryo transfer.

P-434. The smooth endoplasmic reticulum clusters in oocytes associated with higher estradiol and progesterone and larger follicles

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Introduction: We have previously reported the relationship between smooth endoplasmic reticulum clusters (sERC) in mature human oocytes and low pregnancy outcome. However, the cause of the sERC formation is not yet known. We have also investigated other hormonal indicators and follicle size to search for the cause of the sERC formation.

Materials and methods: Consecutive ICSI and combined IVF and ICSI attempts from November 2000 to May 2002 in our clinic were retrospectively covered by this study. A total of 254 patients were divided into four groups according to serum estradiol (E_2) and progesterone concentrations on the day and 36 h after HCG administration to observe the relationships between the pregnancy rate, miscarriage rate (including biochemical pregnancies) and the rate of the sERC-positive cycle: E_2 (HCG day); f1499 pg/ml, 1500–2999 pg/ml, 3000–4499 pg/ml, g4500 pg/ml, progesterone (HCG day); 0.2–0.4 ng/ml, 0.5–0.7 ng/ml, 0.8–1.0 ng/ml, g1.1 ng/ml, E_2 (36 h after HCG); f499 pg/ml, 500–1499 pg/ml, 1500–2499 pg/ml, g2500 pg/ml, progesterone (36 h after HCG); f1.9 ng/ml, 2.0–5.9 ng/ml, 6.0–9.9 ng/ml, g10.0 ng/ml. Patient's largest follicle sizes were also divided into three groups: f18.0 mm, 18.1–21.0 mm, g21.1 mm. We reduced the level of stimulation and administrated HCG on the days when follicle size was smaller than on previous cycles for 10 sERC-positive patients.

Results: Concerning the hormonal data**,** the miscarriage rate was significantly higher for high E_2 and high progesterone on HCG day and 36 h after HCG. The sERC-positive rate was significantly higher at high E_2 (HCG day) and high progesterone (36 h after HCG) ($P < 0.05$). Regarding follicle size, the sERC-positive rate was significantly higher in larger follicles ($P < 0.05$). Pregnancy rate and patient's age did not show any significant difference in any group. Of all the 10 sERC-positive patients who had reduced stimulation and follicle size, none of their oocyte had sERC. Four out of 10 patients (40.0%) achieved pregnancy and two of those gave birth; the others miscarried.

Conclusions: The presence of sERC could indicate excessive ovarian stimulation and oocyte maturation. The elevated estradiol and progesterone concentrations and larger follicles could induce sERC formation. The sERC could prove to be an important area of research in the study of oocyte maturation and development.

P-435. Dynamic ultrastructure of human blastocyst

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Introduction: The aim of this study was to investigate three-dimensional (3-D) ultrastructural events of human blastocysts after conventional IVF or ICSI by transmission (TEM) and field emission scanning electron microscopy (SEM). This study reveals some relevant aspects of the fine morphology of early human embryogenesis.

Materials and methods: The materials included seven early stages of blastocysts, eight expanding stages of blastocysts and six hatching blastocysts obtained after informed consent of patients. The samples were frozen/cracked and studied by SEM following the osmium-dimethylsulphoxide-osmium method. The specimens fixed with 1% glutaraldehyde in phosphate buffered saline (PBS) were rinsed with PBS. They were immersed in 15%, 30% and 50% dimethylsulphoxide in water for 15 min each. The dried specimens were coated with 3 nm of platinum in an ion coater (E-1030: Hitachi) or with 3 nm of osmium in a hollow-cathode plasma CVD osmium coater (HPC-30; Vacuum Device Inc.), and observed with a SEM (Hitachi S-4500) at 15 kV and a TEM Zeiss M10.

Results: (1) Many spermatozoa were still attached to the zona pellucida of the blastocysts. (2) A black lozenged crystallization was noted inside inner cell mass. (3) The morphological changes of mitochondria on various stages of blastocysts were traced, and it was suggested that mitochondria had significant endowment to the early embryo development. (4) A stereo pair of healthy blastocysts with opening windows on the trophoblast, which was taken by SEM with a tilted angle of $\pm 10°$, was illustrated. (5) Outside views of hatching blastocysts with active filopodia and two cells like polar body adjacent to the inner cell mass were revealed.

Conclusions: Important stages of early human development portrayed with these techniques allowed a real 3-D microtopographical analysis. This has the potential to be a valid guideline for a more correct comparison and evaluation of correlated physiological and pathological events of clinical relevance during IVF.

P-436. The rate of blastocyst formation and development between using three different sequential culture media

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Introduction: The aim of this study was to evaluate the efficacy of the different available commercial culture media in terms of blastocyst formation and development.

Materials and methods: A total of 525 zygotes from 90 IVF treatment cycles were cultured until day 5. They were randomly divided into three experiment groups. Experiment 1: a total of 110 oocytes were retrieved from 39 patients and inseminated by IVF or ICSI. The obtained 109 zygotes were cultured in K-SICM (Cook, Australia) until day 3 and then transferred to K-SIBM (Cook, Australia) until day 5. Experiment 2: a total of 139 oocytes were retrieved from 22 patients and divided into two sibling oocyte groups. The obtained zygotes were cultured in each K-SICM or BAS1 (Blast Assist System 1, Medicult, Denmark) until day 3 and then transferred to K-SIBM or BAS2 (Blast Assist System 2, Medicult, Denmark) until day 5. Experiment 3: a total of 276 oocytes were retrieved from 29 patients and divided into two sibling oocyte groups. The obtained zygotes were cultured in each BAS1 or G1.3 (Vitrolife, Sweden) until day 3 and then transferred to BAS2 or G2.3 until day 5. We transferred blastocysts to patients and defined pregnancy by detection of gestational sac.

Results: The results are summarized in the Table I.

Table I

	Experiment 1	Experiment 2		Experiment 3	
	K-SICM K-SIBM	K-SICM K-SIBM	BAS1 BAS2	BAS1 BAS2	G1.3 G2.3
No. of patients	39	22		29	
No. of treatment cycles	39	22		29	
Average age (± SD)	35.1 ± 5.0	36.8 ± 5.1		33.9 ± 3.3	
Cleavage rate (%)	99.1	98.6	98.6	97.4	96.8
High quality embryo rate on day3 (%)	20.9	13.6	27.0	28.6	36.3
Blastocyst formation rate (%)	42.9	43.1	56.5	48.6	41.9
High quality blstocyst rate on day 5(%)	7.1[b]	9.8[b]	26.1[a]	10.8[b]	9.3[b]
Implantation rate (%)	16.3	17.9		24.7	
Pregnancy rate (%)	35.1	31.5		58.6	

Significant differences between [a] and [b] ($P < 0.05$).

Conclusions: High quality embryo rate on day 3 was improved in BAS1 and G1.3 in comparison with K-SICM. With regards to BAS1–BAS2, the rate of blastocyst formation and high quality blastocyst rates were significantly higher than others. From the three media types experimented with BAS1–BAS2 media lead to a higher rate and quality of blastocyst formation. We recommend BAS1–BAS2 as the most viable choice of media for human IVF.

P-437. Oocyte morphology does not affect day 3 embryo quality after ICSI

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Introduction: There are limited prospective publications about oocyte dysmorphisms and its influence on embryo quality in ICSI. Moreover, the resuls are contradictory and other factors such as maternal age or serum estradiol (E_2) on the day of HCG could influence the outcome. In this work we tried to determine which factors are predictive to achieve good (⩾6 cells and ⩽30% fragmentation) and poor (<6 cells and/or >30% fragmentation) day 3 embryos after ICSI.

Materials and methods: The study consisted of 1330 human oocytes at metaphase II (MII) stage from 256 cycles undergoing ICSI for male infertility during the period from 1 June 2001 to 31 December 2002. Oocytes were retrieved after a long ovarian stimulation protocol. Before ICSI, oocyte morphology was assessed at 400× magnification under Hoffman optics and ICSI procedure was carried out following standard protocols. The quality of the embryos was evaluated on the day of embryo transfer, ~75 h after egg retrieval: 428 oocytes (32.2%) yielded good quality embryos and 902 (67.8%) yielded poor embryos. To identify predictors that may contribute to embryo quality (dependent variable), logistic regression analysis were performed including, as independent variables: female age, serum E_2 on the day of HCG administration, and seven of the major interest dysmorphisms that appear in human oocytes: cytoplasmic vacuoles/inclusions (no/yes), oocyte size (normal, small or large), oocyte shape (spherical, ovoid or deformed), polar body size (normal, small or large), perivitelline space (normal or large), zona pellucida integrity (normal or irregular), and perivitelline debris (no/yes). Adjusted odds Ratios (OR) with 95% confidence intervals were estimated.

Results: The variables analysed were not statistically significant predictors of day 3 embryo quality.

Conclusions: In couples undergoing ICSI, female age, E_2 concentration and oocyte morphology are not associated with day 3 embryo quality. Notwithstanding, the number of cases has to be incremented in order to confirm the results.

P-438. Do the number of available zygotes have a predictive value in the decision to delay embryo transfer?

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Introduction: In recent years it has been a dilemma whether to extend embryo culture, and whether embryo transfer should be performed at 48 or 72 h, thus choosing better embryos to transfer. The purpose of this study was to find out whether the number of zygotes can serve as a predictive value for delaying the embryo transfer from day 2 to day 3, and whether this would enable a better selection of good embryos to be achieved, thereby increasing the pregnancy rates and live birth rates.

Materials and methods: The present study included 222 embryo transfers from our IVF programme from 1999 to 2001. Group A included 111 embryo transfers performed on day 3 (study). Decisions on embryo transfer on day 3 were allocated according to work schedule. The control, group B, consisted of 111 embryo transfers performed on day 2, matched according to superovulation protocol, oocyte retrieval, the conditions of embryo culturing, and having the same number of embryos transferred as the number of embryos transferred in the study group.

Results: When the initial number of zygotes was four or more, and there were at least three embryos available for transfer, there was no difference in pregnancy rates between the groups: 26/71 (37%) in group A versus 28/72 (39%) in group B. However, there was a significant increase in the percentage of live births: 85% (22/26) in the study group versus 64% (18/28) in the control group ($P < 0.04$). When the initial number of zygotes was three or less, pregnancy rates and live births were not different between the two groups.

Conclusion: If the initial number of zygotes is four or more, delaying the transfer of embryos to day 3 is recommended, since it would not reduce the pregnancy rates, but would increase the percentage of live births. When the initial number of zygotes is less then four, it is better to perform the embryo transfer on day 2, since no difference in IVF outcomes was found under such conditions.

P-439. Prognosis of development of zygotes related to the number and distribution of the nucleolar precursor bodies in the pronucleus

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Introduction: Different pronuclear (PN) patterns have been described and related to embryo quality, blastomere multinucleation and cleavage and their potential development to blastocyst. The aim of this study was to establish the best prognosis parameters of zygotes related to the number and distribution of the nucleolar precursor bodies (NPB).

Materials and methods: A total of 569 zygotes were evaluated at 14–23 h post-insemination. Two different classification systems were used to describe polarization and the number of NPB. First, a scoring system described by Tesarik and Greco, allocates zygotes to six different categories (p0–p5). Second, a Z-scoring system described by Scott, included four patterns (Z1–Z4). Embryo selection (day 2) was based exclusively on embryo quality. Embryo morphology and pregnancy rate (PR) were related to zygote scoring.

Results: Distribution of the zygotes in Tesarik's patterns were: p0, 39.7%; p1, 17.8%; p2, 24.1%; p3, 9.8%; p4, 7.7%; and p5, 0.9%. Patterns 0 and 3 had a better prognosis of development and resulted in 60.9% and 67.3% good morphology embryos. High proportion of pattern 4 zygotes developed into multinucleated (14.3%) as well as into bad prognosis embryos (76.2% versus average 44.3%; $P < 0.001$). The distribution of zygotes in Scott's pattern were: Z1, 22.7%; Z2, 6%; Z3, 66.2%; and Z4,

41.1%. Z1 (62.5%) and Z2 (64.7%) zygotes developed into good quality embryos. A high proportion of Z4 zygotes developed into poor quality embryos (51.7%), without statistically significant differences. NPB polarization was independent of the time of observation. No differences were found in PR between transfer with at least one good prognosis PN pattern (Scott's Z1 and Z2 or Tesarik's 0 and 3) and transfers without.

Conclusions: Our results show that Z1 and Z2 or p0 and p3 patterns result in good morphology embryos at a higher rate than the other patterns. No differences were found in the PR between patterns even though the number of uniform transfers was too low to establish conclusions.

P-440. Early cleavage and embryo morphology in ICSI pregnancy outcome

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Introduction: Transfer of early dividing (EDE) and good quality (GQE) embryos resulted in increased pregnancy rates compared with late dividing embryos (LDE) and poor quality embryos (PQE). In this ongoing study we evaluated the correlation between EDE, GQE and blastocyst development, the outcome of EDE and GQE transfer and the effect of transfer route (tubal and uterine) on pregnancy rates.

Materials and methods: For the correlation study 1421 embryos (IVF, gamete intra-Fallopian transfer, ICSI) were evaluated statistically [generalized estimation equation (GEE) model] to ascertain the correlation between EDE, GQE and blastocyst formation. For the pregnancy outcome study 124 ICSI cycles were prospectively evaluated [Mantel-Haezel (MH) test] for the percentage risk difference (RD). The effect of EDE, LDE, GQE, PQE, number of embryos transferred and transfer route on ICSI pregnancy were evaluated. Standard hyperstimulation/ovulation induction and ICSI protocols were followed. Pregnancies were reported as positive β-HCG serum levels and were confirmed with a foetal heart beat (ultrasound) at 7 weeks.

Results: A highly significant ($P < 0.0001$) odds ratio (OR) was found between EDE, GQE and blastocysts (EDE versus GQE: OR 5.8; EDE versus blastocyst: OR 3.0; GQE versus blastocyst: OR 5.1). Factors that showed a significant %RD outcome regarding pregnancy were EDE (18%), GQE (21%) and tubal transfer (17%). Tubal transfer was significantly advantageous for LDE (RD = 19%) and uterine transfer for GQE (RD = 21%). EDE transfer adjusted for number of embryos, GQE and placement had a significant effect on pregnancy rates (RD = 18%).

Conclusion: Early division is a marker of embryo viability and transfer thereof results in increased pregnancy rates. The tubal environment is significantly favourable for LDE and GQE perform well in the uterine and tubal environment.

P-441. Efficacy of hatching stage embryo transfer in IVF-embryo transfer

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Introduction: Recently, advances in human IVF-embryo transfer have been reported using sequential mediums and blastocyst stage embryo transfer. However, in our previous report, using a prospective, randomized study, no advantage was found using the blastocyst stage embryo transfer compared with the conventional day 3 embryo transfer. This study was performed to evaluate implantation and pregnancy rates of hatching stage embryo transfer after multiple failures in assisted reproduction treatment.

Materials and methods: A total of 260 cycles in patients who previously underwent five or more assisted reproduction treatment cycles were evaluated using prospective randomized study. The pregnancy rate and implantation rate was compared between the conventional day 3 embryo transfer (day 3ET: $n = 130$) and hatching stage embryo transfer (hat-ET: $n = 130$).

Results: A pregnancy rate of 20.2% (19/94) and implantation rate of 13.0% (20/154) occurred in the hat-ET, compared with 13.8% (18/130) and 7.5% (20/266) in day-3ET. Among the patients who were 35 years or more, the pregnancy rate of 27.5% (19/69) and implantation rate of 18.7% (20/107) were obtained in hat-ET, compared with 15.2% (22/145) and 9.8% (26/265).

Conclusions: We suspect further blastocyst culture into the hatching stage may aid the final selection of the best embryo in older patients.

P-442. Pronuclear morphology is a valuable criterion for elective embryo transfer

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Introduction: Pronuclear morphology has been proposed as an additional criterion to be used for embryo selection for transfer. Several reports have shown that the zygote grading system used by Tesarik and Greco correlated with pregnancy. Our aim was to evaluate the predictive value of a variant of this grading system and to analyse his correlation with embryo morphology, implantation and pregnancy rate.

Materials and methods: This study was performed between October 2001 and March 2002. All oocytes inseminated (IVF) or injected (ICSI) from our IVF programme were cultured individually in 20 µl droplets of G1 medium under oil. The quality of all 2-pronuclear oocytes (2PN) was evaluated by three observations performed between 15 and 20 h. Zygotes were scored according to the number and distribution of the nucleolar precursor bodies (NPB) within each pronucleus, using a variant of Tesarik's simplified methods. Zygotes were considered morphologically of high quality when: at least 3 NPB were present in each pronuclei; the difference in the number of NPB between the two pronuclei did not exceed 3; and the distribution of the NPB was polarized (all NPB in both pronuclei. were aligned in the adjacent poles): group ZP. Zygotes showing poor quality or abnormal pronuclear patterns were assigned to a single group: Zp. Embryo quality was graded from 1 for the best to 4 for the worst according to the commonly used criteria. Embryo selection for transfer was based solely on embryo quality. Transfers were performed on day 2 or 3.

Results: A total of 157 cycles progressed to embryo transfer stage and were included in the final evaluation. A total of 1767 oocytes were collected, 1255 (71%) of them were fertilized and were scored into the two groups ZP and Zp

Table I. Evolution and quality of embryos according to the zygote polarization

	ZP	Zp	*P*
Number of zygotes (%)	363 (28.9)	892 (71.1)	
Arrested embryos (%)	41 (11.3)	235 (26.3)	0.0001
Good morphology of embryos (%)	158 (43.5)	280 (31.4)	0.0001

Table II. Outcomes according to the type of the transfer

	Transfers with embryos developed from		
	Only ZP	Only Zp	*P*
Transfers	42	73	
Transferred embryos	121	241	
Pregnancies	19	14	
Embryonal sacs	27	17	
Pregnancy rate (%)	45.2	19.2	0.0059
Implantation rate (%)	22.3	7.1	0.0385

Conclusions: Cleaving embryos developing from polarized zygotes have a better evolution (better morphology and less arrested embryos) than embryos developing from abnormal morphology zygotes. This more restricted scoring is highly indicative of outcome after IVF.

P-443. Morphology of the first polar body has no impact on fertilization, embryo quality and pregnancy rates

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Introduction: Recent data suggested that the integrity of the first polar body (1PB) is associated with better rates of fertilization (FR), embryo cleavage (CR), embryo grade and pregnancy (PR). This was retrospectively evaluated in 142 consecutive infertile patients and ICSI cycles.

Materials and methods: Mature MII oocytes were divided into intact and fragmented 1PB groups, and the FR, CR, embryo grade (<25% fragmentation) and PR followed. Selection of embryos for transfer was based on embryo grade, irrespective of the source of the 1PB.

Results: Of 823 MII oocytes, 49% had an intact and 51% a fragmented 1PB. No significant differences were found between the two groups regarding the FR (1PB intact, 72%: 1PB fragmented, 67%). On the contrary there were significant differences in the CR (1PB intact, 97%: 1PB fragmented, 99%) and embryo grade (1PB intact, 75%: 1PB fragmented, 82%). In women <35 years there were significant differences between the two groups regarding the FR (1PB intact, 87%: 1PB fragmented, 65%) and the CR (1PB intact, 97%: 1PB fragmented, 99%), but not in relation to the embryo grade (1PB intact, 74%: 1PB fragmented, 82%). For women >35 years, no significant differences were found in all cases. Embryo transfer (mean: 2.1) was possible in 141 cycles (99%), 109 at day 3 (77%) and 32 at day 5 (23%). The total PR was 36% (51/141), 32% (35/109) at day 3 and 50% (16/32) at day 5 ($P > 0.05$). In 23 cycles all embryos transferred came only from intact or fragmented 1PB groups, but no significant differences were found between the PR (1PB intact, 4/12, 33%: 1PB fragmented, 2/11, 18%).

Conclusions: The morphology of the 1PB bears no relationship to the quality of the oocyte and seems not to be a relevant indicator of the embryo implantation potential. In this context, embryo morphology and development should continue to guide the choice of the better embryos elected for transfer.

P-444. Feasibility of telomerase activity in a single human blastomere as an indicator of early embryo development

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Introduction: Telomerase is a ribonucleoprotein that synthesizes telomeric DNA. It has been shown that various tumours, cell lines, and even normal tissues show considerable differences in telomerase mRNA splicing patterns. Based on the importance of telomerase for cellular meiosis and mitosis, we designed this study for the assessment of telomerase activity in single blastomeres. Our objective was to establish a preliminary data first, so that we can understand better embryo physiology, use it as an indicator for embryonic health, or select a best embryo for uterine transfer.

Materials and methods: Oocytes were obtained from patients undergoing IVF procedure after ovarian stimulation. The fertilized oocytes were cultured for 3 days. Group A consisted of normal cleaving embryos at the 4–8 cell stage, which were surplus and not allocated for uterine transfer. Group B consisted of arrested or fragmented embryos at the same stage, which were considered to be compromised. After removing zona pellucida, the embryos were placed in calcium- and magnesium-free medium for dissociation of blastomeres. RNA extraction, reverse transcription, and hTCS (human telomerase catalytic subnuit) PCR amplication were performed. The amplification resulted in a PCR product size of 145 bp and was separated by 3% gel electrophoresis.

Results: In group A, 20 embryos were donated from 17 patients. During isolation, 17 (15.5%) of the individual blastomeres lysed. Intact blastomeres were analysed for the mRNA expression of hTCS. Of the 93 intact blastomeres evaluated, 84 (90.3%) demonstrated telomerase activity by the expression of 145 bp amplified product. Eight (40%) of the 20 embryos had at least one cell that did not express telomerase activity under this procedure. In group B, 35 embryos were donated from 22 patients. Twenty-nine (22.1%) of the individual blastomeres lysed during isolation. Of the 102 intact blastomeres evaluated, 72 (70.6%) demonstrated telomerase activity. Twenty-two (62.9%) of the 35 embryos had at least one cell that did not express telomerase activity. The difference (90.3% versus 70.6%) in the expression of the amplified product was significant in statistics ($P < 0.05$, χ^2), while the difference (40% versus 62.9%) in the embryos that had at least one cell that did not express telomerase activity was not significant ($P > 0.05$, χ^2).

Conclusions: The limitations of evaluating embryos based on morphological criteria alone are well recognized. Many studies have researched more objective criteria for judging embryo viability and implantation potential, although the results are not satisfactory. Our study was conducted to assess telomerase activity in individual blastomeres, in order to reflect embryo development, select better embryos to transfer, and consequently enhance the pregnancy rate without increasing multiple pregnancies. Although the difference in the expression of the amplified product from hTCS mRNA was significant, we still could not predict embryo developmental potential solely from our results, because blastomeres from compromised embryos also showed a high incidence of hTCS mRNA expression (70.6%). Further investigations might be necessary to elucidate its clinical application.

P-445. Prognostic value of first polar body morphology on chromosome aneuploidies in human oocytes

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Introduction: The aim of this study was to investigate if there is a correlation between the morphology of the first polar body of human oocytes and chromosomal aneuploidies, in order to find a non-invasive tool for embryo screening and selection in IVF.

Materials and methods: Metaphase II oocytes that failed to fertilize after IVF or ICSI were fixed and hybridized by fluorescence in-situ hybridization (FISH) with probes for chromosomes 13, 16, 18, 21 and 22 (Vysis). Aneupoidies were detected if non-disjunction or unbalanced predivision were found. Results were correlated to the morphology of the first polar body (Ebener): group 1, ovoid or round, smooth surface; group 2, rough surface; group 3, fragmented; group 4, big or small.

Results: From 98 patients 298 oocytes were fixed; 216 oocytes were at metaphase II, the rest degenerated, in interphase or were lost. A total of 170 oocytes were well spread; 128 well spread metaphase II oocytes were analysed by FISH, yielding 119 results that could be analysed. Of these, 112 were documented with first polar body morphology. In group 1 15% (5/34) were aneuploid, in group 2 17% (4/23), in group 3 30% (11/37) and in group 4 61% (11/18). The difference between groups 1 and 4 was statistically significant ($P < 0.001$).

Conclusions: Human oocytes which failed to fertilize in IVF or ICSI but show a morphological ideal first polar body are chromosomally mostly normal. If their first polar body is big or small, the oocyte is mostly aneuploid. These oocytes should not be first choise for embryo transfer.

P-446. A comparison between a conventional medium and a new generation of sequential media (GIII) for transfer of early cleavage stage embryos

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Introduction: Development of new sequential media has been important to improve the success rate of blastocyst culture. Less interest has been devoted to the development of media used for early cleavage stage embryos. The aim of this study was to compare early embryonic development and implantation rates using an old conventional medium, the human tubal fluid-based IVF-20 medium, and the new GIII-Series developed to meet the demands of the embryo at different developmental stages.

Materials and methods: A prospective, controlled, semi-randomized study has been started in patients undergoing IVF/ICSI. Inclusion criteria were female or male factor infertility, female age below 39 years, and no more than three previous attempts. Informed consent was obtained from all patients and the study was approved by the local ethics committee. Alternate media (G-III or IVF-20) were used during 2–4-week-periods for all patients. Following down-regulation by a long-protocol agonist (nafarelin), patients were stimulated using recombinant FSH. A maximum of two embryos were transferred, patients below 36 years of age with good quality embryos were advised to replace only one embryo (elective single embryo transfer, eSET), according to the new Swedish law on the practice of assisted reproduction treatment.

Results: During the first part of this study 134 patients were included, 41 in the GIII-group and 93 in the IVF-20 group. The mean number of oocytes did not differ significantly between the groups (8.4 versus 9.9 oocytes) and fertilization rate (62%) and the mean number of embryos selected for transfer (1.6) was the same in the two groups. The ongoing PR (40% in G-III versus 35% in IVF-20) and ongoing IR (26% versus 25%) were high in both groups. With two embryos transferred the PR were 43% and 39%, respectively, and the proportion of twin pregnancies were 15% versus 26%. A high proportion (25% versus 33%) of transfers were eSET and the ongoing PR in these groups were 30% versus 28%, with no multiple pregnancies.

Conclusions: This interim analysis suggests that the sequential G-III media seems to yield embryos of high implantation potential on day 3. In the light of urgent needs to minimize multiple pregnancy, the use of G-III might facilitate the practice of eSET to avoid this complication.

ART - laboratory: ICSI, MESA, TESE

P-447. IVF outcome is related to sperm DNA fragmentation but not to apoptosis

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Introduction: The quality of gametes used in IVF is one of the most important factors for the successful treatment of patients. The influence of conventional sperm parameters on IVF results was overcome by the introduction of ICSI in the 1990s. However, sperm with genetic or chromatin abnormalities cannot be identified during ICSI and are therefore able to reduce the outcome of IVF. Sperm can have aneuploidia, disturbed chromatin condensation or the DNA can be damaged by internal or external factors such as apoptosis or reactive oxygen species (ROS), respectively.

Materials and methods: We analysed the semen of 249 patients who underwent IVF treatment. Sperm DNA damage was analysed by staining with acridin orange (AO), DNA fragmentation by means of the TdT-mediated dUDP nick-end labelling (TUNEL) assay and apoptosis by labelling of the sperm with the apoptosis markers anti-Fas and annexin V. According to the cut-off value after ROC analysis of the parameters analysed, patients were grouped and the results were compared with the outcome of IVF.

Results: By using acridin orange, a marker for denaturated DNA, and TUNEL assay, a method for detecting DNA fragmentation, we found significant lower pregnancy rates in cases of semen with a high percentage of marked sperm (AO: 24.6% versus 37.4%, $P = 0.0156$; TUNEL: 19.0% versus 34.6%, $P = 0.0344$). No differences could be detected in fertilization rates. By use of the apoptose markers, annexin V and anti-Fas, no such differences could be observed, neither for fertilization ($P = 0.6106$; $P = 0.6042$), nor for pregnancy ($P = 0.0803$). Age of the women, stimulation protocols and number of oocytes were similar in each group ($P > 0.2$).

Discussion: Our data demonstrated the influence of disturbed DNA integrity on IVF results. Sperm with fragmented or denaturated DNA are still able to fertilize an oocyte. However, at the time when paternal genes are switched on, further embryonic development is disturbed and results in failed pregnancies. Since anti-Fas and annexin V showed no differences in pregnancy rates, apoptosis in the sperm seems not to play a role in IVF. On the contrary, DNA fragmentation in human sperm appears rather to be caused by external factors like ROS, possibly released by leukocytes.

P-448. Frozen-thawed epididymal versus testicular spermatozoa for ICSI in subsequent cycles within the same patients

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Introduction: With the introduction of ICSI, high fertilization and pregnancy rates have been obtained with epididymal sperm. Testicular sperm extraction (TESE) was introduced as an alternative to micro-surgical epididymal sperm aspiration (MESA) in obstructive azoospermia. Although MESA often yields a higher concentration and motility, TESE is carried out in preference to MESA because it is a simpler method and can be carried out under local anaesthesia. However, comparative data on fertilization, pregnancy and embryo implantation rates are sparse and, moreover, are not unequivocal. Since we had the impression that ICSI with frozen-thawed epididymal sperm in our setting was not giving satisfactory results, we analysed retrospectively a consecutive case series within the same patients to compare the results after ICSI with epididymal and testicular sperm frozen in the same elective sperm recovery procedure.

Materials and methods: Twenty-nine couples underwent a MESA-TESE procedure between January 2000 and November 2002. This was in an elective setting, and testicular as well as epididymal spermatozoa were recovered and cryopreserved. On the day of oocyte retrieval, epididymal or testicular spermatozoa were thawed. In all cases, epididymal sperm was thawed in a first cycle and testicular sperm in the following cycle.

Results: Fertilization rates following ICSI with frozen epididymal (72.6%) versus testicular spermatozoa (72.7%) were comparable. Unipronuclear fertilization rate was higher following ICSI with testicular spermatozoa (9.9%) than with epididymal spermatozoa (5.8%). The proportion of good quality embryos was significantly higher following ICSI with epididymal (64.7%) versus testicular sperm (55.4%). Ongoing pregnancy rate was significantly higher using frozen testicular (31.0%) versus epididymal sperm (6.9%). Although the number of transferred embryos was significantly higher (2.0) in ICSI with testicular sperm, the ongoing implantation rate per embryo was also significantly higher.

Table I

	Sperm source		
	Frozen epididymal	Frozen testicular	
No.of cycles 29	29		
No. of transfers	29	29	
Mean female age ± SD	32.8 ± 4.8	33.4 ± 5.0	NS (*t*-test)
No. of succesfully injected MII oocytes	285	290	
No. of 2 PN (% of MII)	207 (72.6)	211 (72.7)	NS (χ^2)
No. of 1PN (% of MII)	12 (5.8%)	21 (9.9%)	NS (χ^2)
No. of embryos for ET and cryo (%)	134 (64.7)	117 (55.4)	$P=0.03$ (χ^2)
No. of embryos transferred	49	58	
Mean no. of embryos transferred ± SD	1.7 ± 0.6	2.0 ± 0.5	$P=0.04$ (t-test)
No. of positive HCG	4	11	
% per transfer	13.8	37.9	NS (Fisher's exact test)
No. of ongoing pregnancies	2	9	
% per transfer	6.9	31.0	$P=0.04$ (Fisher's exact test)
No. of ongoing implantations	2	10	
% per transferred embryo	4.1	17.2	$P=0.03$ (Fisher's exact test)

Conclusion: After elective sperm recovery and cryopreservation in obstructive azoospermia, ICSI with frozen-thawed testicular sperm in a second cycle reveals significantly higher ongoing pregnancy and individual embryo implantation rates than with frozen-thawed epididymal sperm in a first cycle.

P-449. Large perivitelline space decreases fertilization after ICSI

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Introduction: Oocyte features and their influence on fertilization are controversial in ICSI. Moreover, other factors such as maternal age, ovarian stimulation protocol or estradiol (E_2) concentration on the day of HCG administration could influence the outcome. Therefore, the aim of this work was to identify which factors may contribute to the success of fertilization after ICSI.

Materials and methods: We have analysed 5974 human oocytes at MII stage from 517 cycles undergoing ICSI during the period from 1 June 2001 to 31 December 2002 at our Centre. Of these oocytes, 4568 were from infertile patients (401 cycles), and 1406 from donor women (117 cycles). To identify predictors of fertilization rate (dependent variable), we used logistic regression analysis including as independent variables: female age, stimulation protocol (short/long), serum E_2 on the day of HCG administration, and the major interest oocyte dysmorphisms: granular cytoplasm (normal/severe), cytoplasm vacuoles/inclusions (no/yes), oocyte size (normal, small or large), oocyte shape (spherical, ovoid or deformed), polar body size (normal, small or large), perivitelline space (normal or large), zona pellucida integrity (normal or irregular), and perivitelline debris (no/yes). Before ICSI, oocyte morphology was assessed at 400× magnification under Hoffman optics and ICSI procedure was performed following standard protocol. We estimated adjusted odds ratios (OR) with 95% confidence intervals.

Results: Only one significant predictor of fertilization was identified in both groups of patients: perivitelline space. The normal fertilization rate among those with large perivitelline space was 58.3%, lower than that observed among those with normal one, 64.0%. We found a statistically significant association between large perivitelline space and lower fertilization rate ($P=0.003$), OR = 0.78 (0.66–0.92). Female age, stimulation protocol, estradiol concentration, and the other variables of oocyte dysmorphisms were not significant predictors of fertilization after ICSI. Main results remained unchangeable when the analysis was repeated by patient origin (infertile patient or donor).

Conclusions: According to our results, the assessment of the perivitelline space before ICSI could be a valuable means of achieving better fertilization rates independently of the oocyte origin (infertile patient or donor).

P-450. Results of 219 IVF-ICSI cycles in serodiscordant couples (seropositive men) to HIV-1

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Introduction: The indication of IVF-ICSI in HIV-1 serodiscordant couples are the same as in other couples: tubal obstruction, insufficient seminal quality for doing arttificial insemination (AI), and failure of three AI cycles. We show the results of IVF-ICSI cycles made on 156 HIV serodiscordant couples treated in two centres: Instituto de Reproducción CEFER (Barcelona) and ESMAN Medical Consultino (Milan).

Materials and methods: All male patients were accepted independently of stage of viral infection. The sperm was washed through PureSperm gradients centrifugation and swim-up. An aliquot of motile spermatozoa was used to test HIV-1 (RNA and DNA) by PCR technique. Previously spermatic decondensation was made with dithiotheitol. The other aliquot of motile spermatozoa was frozen in liquid nitrogen until the day of follicular puncture. The ovary stimulation, follicular puncture, and ICSI technique were as in other patients. The presence of HIV-1 antibodies was investigated in woman after embryo transfer. Some embryos were frozen if it was indicated.

Results: The number of couples treated was 156: 219 ICSI cycles, 1.4 cycles/couples. The total number of pregnancies obtained with fresh and frozen embryos was 92: pregnancy/cycle, 42.0%; pregnancy/woman, 59.0%; spontaneous miscarriage, 25 (27.2% of pregnancies). There were 58 deliveries and 75 babies were born. The number of ongoing pregnancies is nine. No woman was seroconverted to HIV-1.

Conclusions: The clinical results demonstrate that no seroconversion of the female patients occurs, either because there is no virus or else because the inoculum transferred is so small that it cannot be transmitted. In the IVF-ICSI cases one single spermatozoa, the theoretical vehicle of the virus, is used per oocyte. The IVF-ICSI results obtained from this group of patients are better than those from sub-fertile patients; this is attributed to the fact that serodiscordant couples are fertile and the average female age is lower. We consider IVF-ICSI with washed semen to be a valid technique when it is indicated to help seropositive men to have their own children without transmitting the virus to their partners or to their offspring.

P-451. Pentoxifylline: an effective tool for ICSI with testicular sperm

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Introduction: Testicular sperm have been used successfully for treating men with azoospermia. Freezing of testicular spermatozoa avoid unnecessary ovarian stimulation to oocyte retrieval or minimize the number of surgical procedures on the testis. However, considerable time is required for isolating an appropriate spermatozoon, due to low numbers combined with impaired motility after thawing. These spermatozoa unrelated factors could be a negative impact on final outcome. In trying to avoid these factors, we developed a simple and fast method based on the activation of spermatozoa motility using pentoxifylline in the final testicular sperm concentrate.

Materials and methods: We made a retrospective study including 54 cycles of 39 patients who underwent conventional testicular sperm extraction (TESE). The samples were frozen previously to initiate the ovarian stimulation. The samples was frozen according to routine protocol with TEST Yolk Buffer (Irvine Scientific, USA). Ovarian stimulation, follicular puncture and oocytes preparation for ICSI were performed according to standard methods. After oocyte retrieval, testicular specimen was thawed and centrifuged in a microcentrifuge tube. The addition of pentoxifylline (5 mM) was performed directly in a drop of polyvinylprrolidone (PVP)-free medium, where previously we had added a small volume of pellet. Because spermatozoa motility was produced in short time (2–3 min), a period of incubation or special conditions was not necessary. Spermatozoa swam-out of the drop and was harvested from interphase medium-oil. It was then transferred to a PVP-medium drop where it was washed and selected.

Results: A total of 459 oocytes were microinjected, obtaining normal fertilization in 66.6%. Embryo development was observed in 96.8% of fertilized oocytes. Embryo transfer was performed in all cycles (2.5 ± 0.1) and superfluous embryos were frozen from 100 of them. The implantation rate was 18.7%, resulting in a total of 21 pregnancies (38.8%) with three abortions (14.3%). To date, eight healthy children have been born.

Conclusions: The in-situ use of pentoxifylline to assess testicular spermatozoa motility after thawing is an effective tool. This method offers a good alternative to the controversy that exists in the freezing-thawing of testicular spermatozoa.

P-452. Preliminary results of the use of laser on immotile spermatozoa (from ejaculate and biopsy) on the fertilization rates

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Introduction: Immotile spermatozoa is the usual form we observe when the source of sperm is testicular or epididimal [testicular sperm aspiration (TESA), testicular sperm extraction (TESE), micro-epididymal sperm extraction (MESE) or micro-epididymal sperm aspiration (MESA)] even sometimes in fresh ejaculate. We compared the fertilization rates of immotile but viable spermatozoa after ICSI procedure.

Materials and methods: Patients whom submitted to our assisted reproduction treatment unit for treatment with ICSI entered the study. Group 1 ($n = 16$) was classified as fresh ejaculate sourced from immotile spermatozoa, while goup 2 ($n = 12$) had immotile spermatozoa sourced from fresh testicular or epididimal biopsy or freeze-thawed. Spermatozoa of group 1 was prepared by swim-up method after two-staged centrifugation (15 min of 1100, 900 rpm). ICSI dishes were prepared and oocytes were placed in 5 µl droplets while spermatozoa were placed in 30 µl pool. In the pool, with the help of the pointer, spermatozoa were shot for 1.1 and 1.2 ms, respectively, for group 1 and 2, with a laser (1.48 µm diode laser system) on the tip of their tail (Fertilase, MTM, Montreux, Lausanne, Switzerland). When the spermatozoa tail showed a slow curving reaction to the shooting it was selected for injection and the ICSI was completed. During all procedures we used VitroLife IVF culture mediums. At 16–19 h after injection PN control was done.

Results: For groups 1 and 2 the fertilization rates were 65% and 45%, respectively. There was no morphological difference in the development and cleavage features (fragmentation, blastomer symmetry). The day 13 serum HCG > 10 U were 31% to 22%, respectively, for groups 1 and 2.

Conclusions: Laser applications can be used safely for zona dissection, polar body biopsy, spermatozoa immobilization and permeabilization. The HOS test can be used for selecting viable immotile spermatozoa for ICSI but it is time consuming. Using a laser for sperm selection is very easy and gives a faster handling and hence is a good alternative.

ART - laboratory: IVM

P-453. Importance of estradiol priming in in-vitro maturation cycles

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Introduction: The aim of this study was to evaluate the role of estradiol priming associated or not with gonadotrophins priming in in-vitro maturation (IVM) cycles.

Materials and methods: Twenty-seven patients were allocated to the following three groups. Group 1, 11 patients with a 5 day estradiol priming (4 mg estradiol valerate per day) before follicle puncture; group 2, 8 patients with a 5 day estradiol and gonadotrophins priming (4 mg estradiol valerate plus 150 IU HMG or FSH per day; group 3, 8 patients without any priming. All patients received an HCG injection (5000 IU) 36 h before oocyte retrieval. The latter was performed using specially designed needles (KOPS1235Wood, Cook, Queensland, Australia) with a pressure of aspiration of −7.5 kPa. Immature oocytes were cultured for 24–48 h in TCM199 with gonadotrophins, sodium pyruvate, antibiotics and patient's serum. Mature oocytes were injected and cultured until day 2 or 3.

Results: More oocytes that reinitiated meiosis (metaphase I and II) were retrieved in group 2 than in groups 1 and 3 (13.3, 7.1 and 6.2%, respectively). However, maturation rates were similar among the three groups (66.1, 69.7 and 63.8%, respectively). On the other hand, fertilization rate was statistically lower in group 3 than in group 2 (49.3 % versus 83.3 %; $P < 0.001$). It was also lower in group 1 than in group 2 (64.2 % versus 83.3 %; $P = 0.051$). No significative difference was found in the fertilization rate between groups 3 and 1, even if the fertilization rate in group 1 was higher than that observed in group 3 (64.2 versus 49.3%; $P = 0.148$). Cleavage rates were not statistically different among the three groups.

Conclusions: In this study, we demonstrated that the association of a gonadotrophin and an estradiol priming improves fertilization rate in comparison with IVM without any priming. Moreover, estradiol priming alone seems to improve fertilization rates.

P-454. Ultrastructural study of human oocytes in prophase arrest

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Introduction: Previous reports have shown an unexplained arrest of human oocyte maduration after controlled ovarian stimulation in an IVF programme. The main objective of this work was to describe possible subcellular and biochemical abnormalities in prophase I human oocytes.

Materials and methods: For this study were selected two patients included in the IVF programme of the La Fe Hospital. In both cases, after controlled ovarian stimulation, the recovered oocytes (12 and 5) had a madurative blockage at prophase I stage. All prophase I oocytes retrieved failed to mature after 48 h culture. Arrested PI oocytes were fixed in 0.2 % glutaraldehyde. The oocytes were processed for ultrastructural studies.

Results: The zona pellucida (ZP) of studied immature oocytes had abnormal characteristics. The zona is disorganized and thinner than ZP of normal metaphase II oocytes. The outer region of ZP, close to the cumulus cells, looks disjointed in the arrested prophase I oocytes. Unknown structures are found in the periviteline space. Abnormal structures in organelles, such as Golgi complex, mitochondrion and

cortical granules, are not detected in immature oocytes. However, a subpopulation of immature blocked oocytes with large and empty vacuoles can be distinguished. Around these large vacuoles are disposed small vesicles similar to cortical granules. Moreover the ZP of abnormal PI oocyte subpopulation is thinner and more disrupted than the ZP of the rest of the studied oocytes.

Conclusions: We were able to describe ultrastructural zona pellucida abnormalities in arrested prophase I oocytes. A subpopulation of prophase I oocytes with large cytosolic vacuoles with small vesicles around them was found. All these cellular abnormalities could be related with irregular oocyte development in the ovarian follicles.

P-455. Blastocyst development after in-vitro maturation of germinal vesicle oocytes retrieved from stimulated polycystic ovary syndrome patients

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Introduction: In polycystic ovary syndrome (PCOS) patients undergoing IVF, a considerable number of immature oocytes are frequently retrieved. In-vitro maturation (IVM) of immature oocytes in these patients represents a potential opportunity of achieving pre-embryos compatible for transfer. The aim of this study was to investigate the developmental competence of germinal vesicle (GV) oocytes following IVM.

Materials and methods: Thirty six PCOS patients who underwent 63 ICSI cycles were included in this study. After retrieval, oocyte maturity was evaluated. Cumulus-corona removed mature oocytes were injected immediately and transferred to the uterus or freezed after development. Seventy-six GV cumulus-corona removed oocytes were cultured in P1 medium for 30 h. The IVM metaphase II (MII) oocytes were immediately injected. The developed zygotes were cultured in PI medium for 2 days. The growing embryos were transferred to a blastocyst medium for additional 3 days. Development into morulae and blastocysts was evaluated on day 5 and then cryopreserved.

Results: A total of 752 oocytes were retrieved and 574 metaphase II (MII) stage oocytes were immediately injected. Thirty-five (48.6%, 35/76) GV oocytes matured *in vitro*. Fifteen (42.8%) were fertilized. On the day 5, six morulae (40.0%) and three blastocysts (20.0%) were achieved. Five embryos were arrested: one in the 2-cell stage, two in 4-cell, one in 6-cell, one in 8-cell and one at 2PN.

Conclusions: IVM GV oocytes from stimulated cycles in PCOS patients retain an developmental potential up to the blastocyst and morulae stage on day 5. These immature oocytes can provide a valuable source of embryos for the future use of an infertile couple. Although about half of the GV oocytes had matured *in vitro*, only 20.0% of the matured oocytes, have reached the blastocyst stage. This low blastocyst development can be explained by the absence of cumulus-corona cells during IVM.

ART - laboratory: procedures

P-456. The utility of autologous endometrial coculture after failed IVF: a report on 1194 consecutive IVF cycles from a single institution

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Introduction: The success after IVF depends on many factors. Failed IVF attempts are negatively associated with subsequent IVF outcome. Another critical factor of IVF success involves the quality of the in-vitro culture conditions. We have developed a unique coculture system utilizing the patient's own endometrial cells and successfully applied this to our clinical IVF-embryo transfer programme. In a previous randomized trial we demonstrated the significant beneficial effects of autologous endometrial coculture (AECC) for the growth of human embryos. Embryos grown in AECC were of better quality (more blastomeres, less cytoplasmic fragmentation) than those grown in conventional media. In this study, we analysed the effectiveness of AECC in improving embryo quality and pregnancy rates in 1194 consecutive cycles of IVF-embryo transfer utilizing AECC from January 1996 to December 2002.

Materials and methods: Embryos from each of 1194 patients allocated to growth on AECC were analysed for outcome. All patients had previously undergone failed IVF cycles. During a luteal phase biopsy (5–12 days after LH surge) made prior to the treatment cycle, glandular (G) and stromal (S) endometrial cells were isolated by enzymatic digestion and separated based on differential sedimentation rates. These cells were cryopreserved, then plated as a 50%/50% combination of G and S cells prior to embryo exposure. The conditioned medium (CM) was changed every 2 days. Embryos were randomly grown on AECC or conventional media if more than six oocytes normally fertilized. Otherwise, all embryos were grown on AECC.

Results: The mean age of the patients was 36.88 $\pm$ 3.9 years with an average number of 3.0 $\pm$ 1.8 failed IVF cycles. An overall pregnancy rate of 50.6% and an overall clinical pregnancy (positive fetal heart) of 42.4% were found. The implantation rate was 16.8%. Maternal age was the most important predictor of outcome. Table I demonstrates the age-based IVF outcome with AECC. There were no incidents of contamination or infection related to the AECC procedure.

Table I

	Age <35 years (n = 336)	Age 35–39 years (n = 521)	Age 40–45 years (n = 337)
Previous failed cycles	2.55 (1.4)	3.1 (1.4)	3.3 (2.1)
Mean no. ET	3.2 (0.9)	3.5 (1.1)	4.0 (1.7)
Pregnancy (%)	59.5	53.2	37.7
Clinical pregnancy (%)	51.5	44.9	29.4
Implantation rate (%)	25.4	17.1	7.9

Conclusions: In this large report of IVF outcome, we demonstrated that AECC is an effective tool in the treatment of patients with multiple failed IVF attempts. AECC appears to be a useful adjunct in the treatment of patients with multiple failed IVF attempts and/or poor embryo quality.

P-457. Effect of oxygen concentration on the culture of human embryos: a prospective, randomized study

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Introduction: It has been shown that embryos from several species (mouse, goat, sheep, and cattle) improve their development if cultured under lower oxygen (O_2) concentration. For human IVF the embryo culture is reported to be performed using a gas phase containing either atmospheric (~20%) or reduced (~5%) O_2 concentrations. The literature is nevertheless still not clear about the possible toxic effect of O_2 concentration on human embryo development. This study was therefore undertaken to test the effect of O_2 concentration on human in-vitro embryo development until day 3 (cleavage, fragmentation, multinucleation and compaction rates are analysed) and implantation potential.

Materials and methods: One hundred and forty unselected patients undergoing IVF/ICSI treatment in our centre were randomly allocated at the time of HCG administration to either culture in IVF/RS1 media (Vitrolife) under 5% O_2 concentration (study group) or IVF/RS1 media (Vitrolife) under atmospheric O_2 concentration (control group) using 35 µl droplets under oil.

Results:

Table I.

	Control group	Study group	*P*
Cycles	70	70	
Age (mean ± SD)	33.4 ± 4.1	33.7 ± 3.47	NS
No. of CCOCs retrieved (mean ± SD)	12.9 ± 5.5	13.3 ± 4.1	NS
No. of matured oocytes (mean ± SD)	10.1 ± 3.8	11.7 ± 3.5	NS
Fertilization rate (%)	71.6	69.6	NS
Cleavage rate (%)	97.2	98.9	NS
No. of cells in day 2 (mean ± SD)	3.0 ± 1.4	3.1 + 1.2	NS
Percentage of fragments in day 2 (mean ± SD)	14.9 ± 7.9	20.1 + 7.1	NS
Percentage of multinucleated embryos (mean ± SD)	3.8 ± 0.4	4.3 + 0.3	NS
No. of cells in day 3 (mean ± SD)	5.1 ± 2.1	5.3 ± 2.2	NS
Percentage of fragments in day 3 (mean ± SD)	18.2 + 5.2	21.1 ± 6.1	NS
Percentage of compacted embryos (mean ± SD)	4.6 ± 0.2	2.8 ± 0.3	NS
No. of embryos transferred (mean ± SD)	2.6 ± 1.1	2.7 ± 1.0	NS
Implantation rate (sacs/embryo transferred)	14.8	18.6	NS

Conclusions: This study failed to demonstrate any correlation between oxygen concentration in the culture medium and embryo morphology and developmental speed. Furthermore, the in-vitro culture in 5% O_2 until day 3 do not lead to an improvement in the embryo implantation rate. It remains to be determined if low O_2 concentration will have a beneficial effect during the later stages of preimplantation development (from day 3 to blastocyst stage).

P-458. Successful moving of an IVF laboratory with air quality monitoring

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Introduction: It is known that oocytes and embryos are extremely sensitive to environmental influences, in particular the volatile organic compounds (VOCs). Proper case-control studies, however, are not available for obvious reasons. In summer 2002 our department, including the IVF unit, had to move into newly built premises. Organic solvents are widely used in the building (paints, glue). In order to monitor the anticipated initial reduction in fertilization and pregnancy rates (FR, PR) often observed after moving or other changes, we decided to quantitate VOCs in the laboratory air before (L1) and after (L2) the move.

Materials and methods: VOCs were determined in the laboratory air, close to the working space and incubators, by passive sampling onto active charcoal over 2 week periods, followed by extraction and chromatogrphic-mass spectroscopic analysis. Aldehydes were collected by pumping a defined volume of air through a dinitrophenyl hydrazinone cartridge. Extractions and analyses were performed by an external environmental chemistry laboratory. The VOC levels were set against the IVF results of embryo scoring, fertilization and pregnancy rates. Forty-seven oocyte retrievals were performed in L1 and 30 in L2.

Results: Thirty-five polar and 35 apolar VOCs, 13 aldehydes and acetone were determined. Of these 84 substances, the levels of 74 were found to be very low (<10 $\mu g/m^3$) or undetectable in L1, and 80 in L2. Alcohols and acetone showed the highest levels in both locations, plus some aldehydes in L1 only. Fertilization rates were 62% and 68%, and pregnancy rates per embryo transfer were 34.2% in L1 and 32.1% in L2, respectively. Cumulated embryo scores were 39.4 in L1 and 42.1 in L2.

Conclusion: In this prospectively planned study, no differences were seen between L1 and L2 for any clinical IVF parameter while the air quality was found to be better in L2. Alcohols (hospital disinfectants) did not affect the outcome, neither did the measured levels of formaldehyde and acetone since the pregnancy rates were equally good at both locations. The measurement of organic substances in the air is very sensitive and can be recommended when changes are made to a laboratory or when the FR/PR unexpectedly decrease.

P-459. Development and evaluation of a RT-nested PCR protocol for viral validation of HIV processed semen

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Introduction: Assisted reproduction treatment could help HIV serodiscordant couples to have children minimizing the risk of HIV transmission. We developped a method to validate processed semen of HIV seropositive men in assisted reproduction of serodiscordant couples.

Materials and methods: We developed a RT-nested PCR amplifying a sequence of the HIV pol gene, a highly conserved region in B and non-B HIV strains. The RT-nested PCR was evaluated in HIV positive plasma of known viral load, in arficially infected semen and in 28 semen samples of 16 HIV seropositive volunteers. After liquefaction and examination of semen, a two-gradient (40 and 80% solutions) separation method was applied (Pure Sperm, Nicadon International, Gothenburg, Sweden) for separation of spermatozoa from seminal plasma and non-spermatozoal cells (NSC). An aliquot of fresh semen was also centrifuged to separate seminal plasma and cells pellet. Fresh semen, seminal plasma and cells pellets obtained after centrifugation, selected spermatozoa pellets and supernatants obtained after gradient separation were evaluated for the presence of HIV RNA.

Results: To obtain a good sensitivity four PCR reactions were conducted for each extraction and a result was considered positive when one or more PCR reactions per extracted sample were found positive. Using plasma of HIV positive patients and a quantified HIV positive plasma obtained thanks to the NIH AIDS Research and Reagent programme, the detection limit of our RT-nested PCR was 20 RNA copies/ml. We observed inhibition of RT-nested PCR in arficially infected fresh semen and seminal plasma. Inhibition was suppressed by sample dilution. The detection limit was also 20 RNA copies/ml. Among eight semen samples of eight patients with plasma viral load >500 RNA copies/ml (range 888–50000), we detected HIV RNA in 5/7 fresh semen, 7/7 seminal plasma, 4/5 cells pellets, 3/5 gradient supernatants and in 2/7 selected spermatozoa pellets. Patients with HIV RNA positive spermatozoa had 50 000 and 20 000 HIV RNA copies/ml in their plasma. Among 20 semen samples of seven patients with plasma viral load <500 copies/ml, we detected HIV RNA in 2/20 fresh semen, 3/16 seminal plasma, 0/5 cells pellets, 0/17 gradient supernatants and 0/19 selected spermatozoa pellets. Two patients with a viral load below 50 RNA copies/ml (undetectable in blood) showed positive detection in fresh semen (1/2) and in seminal plasma (2/2).

Conclusions: We developed a sensitive RT-nested PCR for the detection of HIV RNA in different parts of semen preparations for assisted reproduction. The HIV testing of the semen preparation could be done on the same day. Our preliminary results show that HIV RNA could be detected at different steps of the semen preparation, even in the final product. Viral validation of processed semen is indispensable. When using PCR techniques, inhibition of PCR must be controlled using sample dilution and internal control. Quality control assessment should be organized for fertility centres involved in assisted reproduction for HIV serodiscordant couples.

P-460. Short time sperm-oocyte exposure during IVF improves embryo quality

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Introduction: Fertilization takes place very early during IVF. An 18 h incubation is therefore not necessary and may even be deleterious for embryo quality, since reactive oxygen species are produced by cell metabolism and cell death, even though previous reports showed controversial results. To test this hypothesis, a prospective study was set up in which sibling oocytes were randomly allocated to short or standard insemination.

Materials and methods: A total of 108 couples were included in the study irrespective of the indication of the assisted reproduction treatment. All women underwent controlled ovarian hyperstimulation with recombinant FSH. Sperm were prepared by two density Pursperm gradient (90 and 45%) centrifugation. Group A (1 h) and group B (18 h) contained 402 and 442 metaphase II oocytes, respectively. IVF was performed into 20 µl drops of culture medium under oil in a CO_2 5 % atmosphere. All oocytes were inseminated with the same sperm concentration (3000 motile sperm per drop). In group A, cumulus oophorus complex were washed after 1 h of insemination and incubated in a fresh drop of culture medium. Fertilization rates, embryo quality (good embryos contained less than 20% cytoplasmic fragmentation) and number of blastomeres per embryo were evaluated by a logistic statistical test using the generalized estimating equations, taking into account results observed for each patient in the two groups.

Results: Fertilization rates were not statistically different, 65.7 % and 67.9 % for groups A and B, respectively. However, the embryo quality was statistically better in group A than in group B at day 2. Indeed, the percentages of good embryos were 60.7 % and 52.0 %, respectively ($P = 0.03$, OD 1.44, 95% CI 1.00–2.01). The mean number of blastomeres per embryo evaluated by a paired Wilcoxon test was not different (4.67 versus 4.84; $P = 0.54$).

Conclusion: The present study showed that a brief gamete co-incubation lead to an improved embryos quality without any detrimental effect on the fertilization rate. It demonstrates that short gamete incubation period could reduce the negative effect induced by degenerative cells during standard IVF procedures.

P-461. Artificial oocyte activation as a routine in human assisted reproduction laboratories

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Introduction: The major reason for failed fertilization after ICSI seems to be related to oocyte activation failure, due to intrinsic factors in oocytes and/or in sperm. Based on observations in which fertilization failures after ICSI can be overcome by artificial increase of the free cellular calcium concentration, ionophore boosted oocyte activation has been applied in assisted reproduction attempts in pre-selected cases of poor prognosis. The present study intended to clarify the real contribution of calcium ionophore (A23187, Sigma) as an artificial oocyte activation (AOA) in ICSI cycles.

Materials and methods: A total of 155 ICSI cycles (125 patients) were included, divided according to indication for AOA: ejaculated spermatozoa from severe male infertility (sperm concentration $<1 \times 10^6$/ml, group A1) and from ovary factor (oocyte retrieved number less than 4, group A2); spermatozoa from testicles (group B); and from epididymis (group C). ICSI was conducted based on normal protocols. Immediately after ICSI, metaphase II (MII) oocytes were maintained in activation medium [15% of substitute synthetic serum (SSS), in human tubal fluid (HTF) plus 1 mM of A23187] for 30 min. Oocytes were transferred to HTF 15% and 17 h after ICSI fertilization was checked. Laboratory data were the main outcome measured and compared with the control (the same indications, but without AOA with A23187).

Results: The results are shown in the Table I.

Table I.

	A1	Control A1	A2	Control A2
Cycles/patients	34/28	51/41	31/30	53/42
Maternal age (SD)	33.9 (6.3)	33.6 (4.9)	37.1 (5.4)	38.8 (4.0)
Normal fertilization	73.1	68.3	70.3	73.9
Fertilization failure	18.1	15.7	8.3	9.1
Pregnancy rate/patient	32.1	27.5	13.3	12.9
Implantation rate	16.1	10.2[a]	7.4	7.1
	B	Control B	C	Control C
Cycles/patients	36/30	154/133	36/30	79/69
Maternal age (SD)	32.6 (5.4)	32.2 (5.5)	32.6 (5.4)	32.7 (5.3)
Normal fertilization	57.2	55.4	65.4	59.9[c]
Fertilization failure	21.7	29.6	17.3	19.8
Pregnancy rate/patient	51.3	25.5[b]	29.0	26.8
Implantation rate	21.0	9.9[b]	13.5	10.6[c]

[a, b, c] $P < 0.05$ ([a]A1 versus Control A1; [b]B versus Control B; [c]C versus Control C).

Conclusions: This treatment did not affect embryo development and pregnancy establishment and seems to improve results in selected patients who have poor quality gametes. We concluded that AOA should be employed as routine in cases with important seminal disorders, mainly when ICSI is applied with spermatozoa from the testicles.

P-462. Importance of laboratory design and quality control in an IVF laboratory

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Introduction: An IVF laboratory requires special conditions to be functional to assure optimal conditions by the means of the quality control: physical requirements, air quality, appropriate equipment and highly qualified personnel. The aim of this study was to evaluate the influence in pregnancy rates of changing quality control variables in an IVF laboratory.

Materials and methods: A total of 446 IVF and ICSI cycles were included, 223 of these cycles were performed in 1 year in one IVF laboratory (Lab A) before we changed to another (Lab B) on 15 May 2001. These cycles were compared to the first 223 cycles done in Lab B. Similar protocols for ovarian stimulation were used and the same embryologists and clinicians participated in both. The media culture and incubators were the same. Lab B was designed to offer the highest air quality, with proper air purification and filtration system, and a stricter quality control programme was established. Fertilization rate, embryo quality, pregnancy, implantation and abortion rates were evaluated in IVF and ICSI cycles done in both laboratories.

Results: We performed 223 cycles in each laboratory, similar number of oocytes were retrieved (12.2 ± 4.1 and 10.8 ± 3.4) and fertilized (68.9% ± 17.2 and 70.5% ± 14.3). Good quality embryos in both laboratories showed no statistical differences (4.2 ± 2.1 and 5.1 ± 2.2), but we found significantly lower media number of blastomeres in embryos of day 2 ($P < 0.05$) in Lab A when compared with B (2.4 ± 1.4 and 3.3 ± 0.8). Pregnancy rates were significantly lower ($P < 0.001$) in Lab A (56/223, 25.1 %.) than in B (96/223, 43.0%) as well as implantation rates (77/847, 9.1% and 123/713, 17.2%; $P < 0.001$), in spite of similar numbers of transferred embryos (3.8 ± 1.1 and 3.2 ± 0.8). Abortion rates showed no differences.

Conclusion: Increasing quality control in an IVF laboratory significantly enhances the implantation and pregnancy rates in an IVF programme.

P-463. Autologous endometrial coculture in patients with IVF failure: correlations of outcome with platelet derived growth factors AB and BB

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Introduction: The development of embryos with monolayers of autologous endometrial coculture (AECC) has been shown to improve outcome for patients with multiple implantation failures after IVF-

embryo transfer. Presumably, this effect is mediated through the production of trophic factors expressed by these helper cell lines. Platelet derived growth factors (PDGF)-AB and -BB are known to be expressed by the endometrium. The specific aim of this study was to determine if PDGF-AB and -BB levels as measured in the supernatants of conditioned media (CM) of embryos cocultured in AECC is predictive of IVF outcome. CM from the ECC cells exposed or non-exposed to human embryos was collected in 42 consecutive patients and assayed for the presence of PDGF-AB and -BB and correlated with outcome.

Materials and methods: During a luteal phase biopsy (5–10 days after LH surge) made prior to the treatment cycle, glandular (G) and stromal (S) endometrial cells were isolated by enzymatic digestion and separated based on differential sedimentation rates. These cells were cryopreserved, then plated as a 50%/50% combination of G and S cells prior to embryo exposure. The conditioned medium (CM) was changed every 2 days. Embryos were randomly grown on AECC or conventional media if more than six oocytes normally fertilized. Otherwise, all embryos were grown on AECC. PDGF-AB and -BB levels were measured utilizing an immunoenzymetric assay. Background levels of PDGF-AB and -BB were also determined from media alone (Hams F-10 supplemented with 15% patient's serum). Statistics included Wilcoxon signed rank test, Mann–Whitney *U*-test and χ^2.

Results: The mean age of the patients was 36.8 ± 3.8 years. They had had on average 3.7 ± 1.9 failed IVF cycles. Twenty-four (57.1%) of the patients had a positive pregnancy test. A clinical pregnancy (positive fetal heart) was demonstrated in 52.4% (22/42) of the patients. PDGF-AB and -BB levels were significantly greater in the CM than in the serum controls. Exposure or non-exposure to an embryo did not result in differing levels of PDGF-AB and -BB in the CM. Embryos grown on AECC demonstrated a significant improvement in number of blastomeres and fragmentation (frag) when compared with embryos grown in conventional media without AECC (6.9 ± 1.3 versus 5.5 ± 1.2 blastomeres and 13.6 ± 9.3% versus 23.4 ± 9.8% frag; $P < 0.05$). PDGF-AB and PDGF-BB levels in the CM were not associated with embryo quality or pregnancy outcome.

Conclusions: We have demonstrated a significant improvement in blastomere number and fragmentation with AECC. The cells in the AECC express PDGF-AB and PDGF-BB; however, the presence of these cytokines was not associated with IVF or AECC outcome.

Contraception: female

P-464. A prospective randomized comparison of sublingual and vaginal misoprostol in termination of pregnancy in the second trimester

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Introduction: The efficacy, side effects and acceptability of sublingual and vaginal misoprostol for second trimester medical abortion were compared.

Materials and methods: Two hundred and twenty-four women at 12–20 weeks of gestation were randomized to receive either sublingual or vaginal misoprostol 400 µg every 3 h for a maximum of five doses. The course of misoprostol was repeated if the woman did not abort within 24 h.

Results: There was no significant difference in the abortion rate at 48 h (sublingual: 90.7%; vaginal: 94.6%). However, the abortion rate at 24 h was significantly higher in the vaginal group (84.7%) compared with the sublingual group (63.6%). There was no difference in the median induction-to-abortion interval (sublingual: 13.8 h; vaginal: 12.0 h). Significantly more women in the sublingual group preferred the route to which they were assigned when compared with the vaginal group. The incidence of fever was also less in the sublingual group.

Conclusion: Both vaginal and sublingual misoprostol were effective for second trimester medical abortion. The use of vaginal misoprostol resulted in a higher abortion rate at 24 h but the abortion rate was similar at 48 h. However, sublingual administration of the drug was more acceptable to women undergoing second trimester medical abortion.

P-465. Follicular activity in women receiving medroxyprogesterone acetate and estradiol cypionate as once monthly contraceptive injection versus a low dose ethinyl estradiol oral contraceptive

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Introduction: Hormonal assays coupled with transvaginal ultrasound are the most important diagnostic methods in the measurement of ovarian activity throughout the menstrual cycle. The aim of this study was to evaluate the effect of a once monthly contraceptive injection containing medroxyprogesterone acetate (MPA 25 mg) and estradiol cypionate (E_2C 5 mg) (Lunelle, Pharmacia & Upjohn, Kalamazoo, USA) versus a low dose (0.02 mg) ethinyl estradiol and levonorgestrel (0.1 mg) oral contraceptive (OC) (Miranova, Schering, Germany).

Materials and methods: In an open-label, randomized, parallel-group multicentre study 18–35-year-old women with intact ovarian function were involved. In this study we have analysed the results of the 22 patients entering the study in our centre. Serum estradiol (E_2), FSH, LH and progesterone levels were determined every second day in the control cycle (C0) and in the following two cycles of treatment (C1 and C2). The patients in group A received an injection of MPA/E_2C at day 1–2 of the C1 and 28 ± 2 days thereafter. In group B patients took the pill each day. Serum E_2, LH, FSH and progesterone levels were compared between the two groups in each cycle. Statistical analysis was performed using Mann–Whitney *U*-test. $P < 0.05$ was considered statistically significant. Results are presented as medians and as 25–75 percentiles.

Results: Demographic and baseline characteristics were comparable between the two groups. In C0 there was no significant difference in the daily LH, E_2 or progesterone levels between the group A and B. On day 24, 26 and 28 a significant difference seen in FSH levels could be attributed to the small number of patients (7 in A versus 4 in B) having spontaneous cycle up to 28 days. Patients in group A in C1 and C2 had a significant increase in E_2 which peaked on day 6 and differed significantly from values in group B [C1: 136 pg/ml (110–144) versus 13.6 pg/ml (13.6–21.8) ($P < 0.001$) and C2: 165.9 pg/ml (136–182.2) versus 36.7 pg/ml (14.3–114.9) ($P < 0.03$)]. MPA/E_2C caused a more persistent and significant suppression of LH (significant on day 6, 10, 26 in C1 and on day 2, 6 in C2) and FSH levels (significant on day 6–28 in C1 and in C2) compared to the C0. Both treatments demonstrated significant suppression of progesterone productionast; in both groups in C1 all progesterone level median- and percentile values were under 1.5 and in C2 values were under 1.0 ng/ml.

Conclusion: Findings from this study suggest that patients taking the pill under well-controlled monitored conditions experience more follicular activity than those treated with MPA/E_2C. The higher E_2 levels in C1 and C2 in group A were attributed to the oestrogen component of the medication. In non-clinical conditions when the daily dosing requirement of the pill and patient compliance play important roles, the residual ovarian activity could be even greater which could cause contraceptive failure.