Conclusions: Presently, no efficient parameters exist to predict sperm retrieval rate in NOA patients, whereas in OA cases sperm retrieval is possible in almost all cases. Therefore, as ICSI outcome regarding fertilization rate is superior in OA patients when using fresh spermatozoa, ICSI should be planned in conjunction with surgical sperm retrieval. In contrast, in patients with NOA, as sperm retrieval is poorer, but ICSI outcomes using fresh or thawed spermatozoa are similar, elective surgical sperm retrieval may be offered to patients prior to ovarian stimulation of their partners. Once non-ejaculated spermatozoa are available for ICSI, only female factors bear a significant impact upon the clinical success rate. Female partners >38 years old and those with less than four mature oocytes in an assisted reproductive treatment cycle have a compromised clinical outcome.

Assisted Reproduction

P-033. The early cleavage of embryos to the two-ceIl stage after ICSI is a good predictor of pregnancy outcome

Mooney C.E, Winston R.M.L, Dawson. K.J

IVF Unit, Hammersmith Hospital, Du Cane Road, London W12 0HS, UK

Introduction: The criteria commonly used for selection of embryos for transfer, are cell number and morphology. A recent study indicates that patients whose embryos are observed to commence cleavage early are more likely to become pregnant. This prospective randomised study provides further evidence to support these findings and further emphasise the importance of maximising the number of early cleaving embryos to improve pregnancy outcome.

Materials and methods: A total of 120 ICSI (Intracytoplasmic sperm injection) cycles each of which resulted at least 6 embryos available for transfer were included in this study. The study its self was purely observational and no positive selection of early cleavage embryos was made, in 57 cycles examination of cleavage was made 27 hours after the ICSI procedure. Embryos that had cleaved to 2 blastomeres were designated 'early cleaving'; those that had not were designated 'no early cleaving'. Selection of embryos for transfer was made on the basis of morphology and on total cell number at the time of transfer and was according to our standard procedures.

Results: The patients in this study fell into one of four groups: (1) A mixture of early and no early cleavage embryos, (2) All no early cleavage embryos (3) The control group where the early cleavage status of the embryo was unknown. There were more ($P = 0.001$ χ^2) clinical pregnancies in group 1 (62.5%) compared with group 2 (44%) Group 3 (34.7%) or group. 4 (33.3%). No differences between the groups were found when comparing key parameters (age, number of embryos available for transfer, number of embryos transferred) of the couples.

Conclusions: This study shows that when patients have only early cleaving embryos transferred, there is an increased chance of a pregnancy when compared to those with a mixture of early and non early cleavage embryos and those with only non early cleaving embryos. These data show the identification of early cleavage as an additional criterion for the selection of embryos for transfer. Therefore a change in protocol to positively select embryos based on their cleavage status is justified. The data suggest the importance of maximising the number of early cleavage embryos for transfer to improve the chances of pregnancy. As a result, an improvement in pregnancy rates might be expected as more patients have transfers consisting of early cleaving embryos.

P-034. Is the use of donor spermatozoa a safe option? Infectious diseases and genetic disorders screening in a sperm donor population and a donor artificial insemination programme: experience from 10 years follow-up

Meseguer M.[1,2]; Garrido N.[1], Zuzuarregui J.L.[1], Romero J.L.[1], Simón C.[1,2], Remohí J.[1,2] and Pellicer A.[1,2,3]

[1]Instituto Valenciano de Infertilidad and [2]Department of Paediatrics, Obstetrics and Gynaecology, Valencia University School of Medicine and [3]Hospital Universitario Dr. Peset, Valencia, Spain

Introduction: Patients undergoing assisted reproduction techniques with sperm donor are often worried about the safety that clinics can offer regarding transmission of infectious diseases and genetic alterations of the samples. Spanish law for assisted reproduction requires periodic blood and semen tests for every candidate and sperm donor, at least 6 months after their last donation, as well as complete anamnesis concerning their familiar history, in order to avoid any transmission of venereal or infectious diseases, and hereditary diseases to the semen recipients and/or offspring. These analyses complicate donor management, and are relatively expensive, so a careful analysis of the situation is mandatory to demonstrate the usefulness and security of the tests. Our aim was to retrospectively assess the incidence and magnitude of infectious diseases or genetic disorders in a sperm donor population, in order to investigate the safety and utility of routine screening for sexually transmitted diseases and genetic disorders in this group, as well as the incidence of infected women artificially inseminated with donor semen and illnesses of newborns from sperm donors that were not detected by their selection.

Methods: A total of 166 donors were included in the study, with mean age 21.9 years (range 18–34). One was black (0.6%) and the remaining were caucasian. They were mainly university students (93.3%), but some of them had finished their college studies (4.8 %), and other situations comprised 1.9%. Blood tests ($n = 552$) consisted in the detection of syphilis (VRDL), hepatitis B antigen, hepatitis C antibodies, antibodies to HIV-1 and -2, herpes virus-1 and -2, cytomegalovirus antibodies, and complete karyotype-based study of their chromosomal baggage. Semen tests ($n = 597$) comprised microbiological culture, to exclude the presence of *Chlamydia*, *Ureaplasma*, *Mycoplasma* and *Gonorrhoea*. Also, complete anamnesis and familial history was evaluated. The women ($n = 218$) studied were intrauterine-inseminated with donor spermatozoa after low dose ovarian stimulation and were questioned about infections and blood tested 6 months after the insemination. Samples were prepared by either swim-up or density gradients. When pregnancy and delivery were achieved, they were asked for anomalies.

Results: No infections in the women were detected in any case (out of 218 artificial inseminations). No cases of inherited diseases transmitted by the male donor were detected in any newborn (out of 50 children). No karyotype alterations were found among the candidates studied ($n = 166$). Among all the candidates, there was one case of convulsive disorder, and another of Steiner's muscular dystrophy and were not accepted by their familiar condition. See Table I.

Table I.

	Positive analysis/total
Infectious blood test	
HIV-1 and -2	0/552
Hepatitis C	1/552 (0.2%)
Herpes virus type I-II	5/552 (0.9%)
Hepatitis B	5/552 (0.9%)
Syphilis	0/552
Cytomegalovirus	4/552 (7.2%)
Infectious semen test	
Total positive cultures	153/597 (25.6%)
Ureaplasma	54/597 (9%)
Mycoplasma	7/597 (0.01%)
Gonorrhoea	1/597 (0.2%)
Chlamydia	5/597 (0.8%)
Other microorganisms	99/597 (16.6%)

Conclusions: Sperm donation is a safe option to be employed in assisted reproduction, since no infection has been described in 10 years at our centre, and no genetic disease has been transmitted to the progeny. Only very infrequent cases of infectious diseases and no karyotype alterations have been found. Nevertheless, a high incidence of microbial infection in semen was described, but mostly irrelevant. Despite the low frequency of significant alterations, routine semen and blood analysis are essential in sperm donors to avoid any spread risk of genetic or infective diseases.

P-035. The effect of combining human serum albumin and hyaluronic acid in the transfer medium on the pregnancy rate in IVF cycles — a prospective randomized study

Chen C.W., Tsai F.P. and Lin J.C.

IVF-Unit, Dr. Tsai & Dr. Chen's Women Hospital, Chang-Hua, Taiwan

Introduction: Human embryos are cultured and transferred in the presence of macromolecules such as human serum albumin (HSA) in order to facilitate embryo manipulation and negate the effects of toxins. Because the fluids of the female genital tract are rather specialized environments for the development of embryos, there is much interest in developing a more defined culture and transfer media. A major component of fallopian tube and uterine fluids are glycosaminoglycans and proteoglycans. Hyaluronic acid (HA), one of glycosaminoglycans is present in the uterus at significant levels. Interestingly, albumin and hyaluronic acid were found to act together in synergy to further increase mouse blastocyst development in culture and also the subsequent viability of mouse blastocysts when it was used as a transfer medium. This study was therefore designed to investigate the effect of HA in the human embryo transfer medium in IVF-embryo transfer cycles.

Materials and methods: Patients undergoing IVF/embryo transfer who were having a day 3 embryo transfers were eligible for this study. Volunteers were randomized to the study group (HA + HSA) or the control group (HSA). In the control group, embryos were transferred in the basal XI HTF with 10% HSA. In the study group, embryos were transferred in the medium with 10% HSA and 0.125 mg/ml HA. The scheduled pregnancy test was performed on the 14th day post embryo transfer.

Results: In this study, embryo transfer was performed in 70 patients. Age, numbers of oocytes, numbers of embryos in both groups did not differ statistically (Table I). Eight pregnancies resulted in the study group (HA + HSA, pregnancy rate 22.8%) compared with five pregnancies in the control group (HSA, pregnancy rate 14.2%). This difference is not statistically significant.

Table I.

	Study group (HAS + HA)	Control group (HSA)
No. of patients	35	35
Mean age	31.35 (22–43)	32.47 (23–40)
Mean no. of oocytes	6.60 (3–13)	7.08 (2–16)
Mean no. of embryos replaced	2.71 (2–5)	3 (1–5)

Conclusions: As the human endometrium and embryo both express the receptor for hyaluronic acid, HA may be involved in the initial phases of blastocyst attachment to the endometrium. The results of this study demonstrate that there is a trend towards a better pregnancy rate with HA added to the transfer medium than the conventional transfer medium but without any statistical significance. Before coming to any conclusion about the effect of HA in the transfer medium, we need a larger randomized, prospective study.

P-036. Modified assisted hatching applied to two-pronuclear stage embryos

Jelinkova L., Paulus W.E., Strehler E., Zhang and Sterzik K.

Fertility Center, Christian Lauritzen Institut, Frauenstrasse 56, Ulm, Germany

Introduction: Assisted hatching (AH) has been used in human reproductive medicine since 1991. However, the indication for AH is still a matter of discussion and the reports about AH are controversial. AH has been applied to day 2 or 3 embryos and/or total zona removal has been used at blastocyst stage. We applied mechanical AH to 2-pronuclear stage embryos, following day 2 or 3 transfer. Relatively large perivitelline space allows X-shaped zona dissection which could prevent a higher incidence of monozygotic twinning. The aim of our study was to evaluate the benefit of this technique to particular age and anamnesis groups of patients.

Materials and methods: A total of 519 patients were involved in this study. The inclusion criteria were: female age ⩾35 years and/or with at least two previously failed embryo transfer attempts. AH was performed 18–20 h after insemination to pre-embryos, selected for transfer. The other fertilized oocytes were cryopreserved. The X-shaped zona dissection was prepared by two steps of partial zona cutting by glass microneedle. After finishing the first slit in zona pellucida, the pre-embryo was rotated so that the first cut was visible at the 6 o'clock position and the second cut was performed at 90° towards the first one. Embryos were cultured for another 1 or 2 days in G1 medium (Scandinavia AB) and than transferred to the patients.

Results: See Table I.

Table I.

Patients	AH group		Control group		*P*
	No. patients	Pregnant[a]	No. patients	Pregnant[a]	
<35 years	40	15 (37.5)	105	22 (20.9)	0.041[b]
>35 years	117	27 (23.0)	257	57 (22.2)	NS[b]
<38 years	95	33 (34.7)	253	60 (23.7)	0.038[b]
>38 years	62	9 (14.5)	109	19 (17.3)	NS[b]
<2 cycles	105	28 (26.7)	157	38 (24.2)	NS[b]
>2 cycles	52	14 (26.9)	205	41 (20.0)	NS[a]
>35 years <2 cycles	59	15 (25.4)	157	38 (24.2)	NS[b]
>35 years >2 cycles	58	12 (20.7)	100	19 (19.0)	NS[b]
>38 years <2 cycles	25	2 (8.0)	65	13 (20)	NS[c]
>38 years >2 cycles	37	7 (18.9)	44	6 (13.6)	NS[c]
<38 years >2 cycles	67	20 (28.9)	65	13 (20.0)	NS[b]
All patients	157	42 (26.7)	362	79 (21.8)	NS[b]

[a]Values in parentheses are pregnancy rate per embryo transferred.
[b]χ^2 test.
[c]Fisher's exact test.

Conclusion: Our data suggest that AH can increase the pregnancy rate per embryo transfer (PR/ET) in patients <38 years. In the category of patients >38 years, probably the other factors, especially the oocyte quality, affect the final PR/ET more than AH. Comparing the categories of patients with less than two cycles and those with at least two previous implantation failures, we observed the increase of the PR/ET in the latter group, but this difference was not statistically significant. No incidence of monozygotic twinning was observed after using the AH modified technique.

P-037. Benefit of vaginal sildenafil in assisted reproduction therapy

Paulus W.E., Strehler E., Zhang M., Jelinkova L. and Sterzik K.

Christian-Lauritzen-Institut, Frauenstraße 56, D-89073 Ulm, Germany

Introduction: Perfusion of uterus and ovaries is an important variable for the success of assisted reproduction therapy. The phosphodiesterase inhibitor sildenafil, known as Viagra, has been used for the treatment of male erectile dysfunction. The vasodilatory effect of sildenafil may also improve perfusion of uterus and ovaries after application to women with poor endometrial response and reduced uterine blood flow.

Materials and methods: In a prospective study, eight patients our fertility centre were treated with vaginal application of sildenafil suppositories (25 mg) four times per day from the third day of the stimulation protocol to the evening before oocyte retrieval after having given their informed consent. Patients were only included after positive screening for reduced uterine artery blood flow (average pulsatility index of left and right uterine artery <2.00) and poor endometrial development (endometrial thickness <8 mm) 1 h before embryo transfer in former therapy cycles. For transvaginal ultrasonography we used a 7.5 MHz transvaginal probe (Logiq 400; GE Medical Systems). In all cases pituitary was down-regulated with nafarelin acetate (Synarela®) in a short protocol starting on the first cycle day and continuing until the day of human chorionic gonadotrophin (HCG) injection. Ovarian stimulation was performed with 2–4 ampoules human menopausal gonadotrophin (Menogon®) beginning on cycle day 3. The dosage was individually adjusted and determined in accordance with previous response. HCG (Pregnesin®) was administered when at least one follicle reached a maximum size of 20 mm and two others 16 mm in diameter. Transvaginal ultrasound-guided needle aspiration of follicular fluid was carried out 36-38 h after HCG administration. Immediately after follicle puncture the oocytes were retrieved, assessed and fertilized *in vitro*. In cases of severe male subfertility, intracytoplasmic sperm injection (ICSI) was performed. The ultrasonography and Doppler examination was repeated just before the embryo transfer. The pulsatility index (PI) for each uterine and ovarian artery was calculated electronically from a smooth curve fitted to the average waveform over three cardiac cycles. Up to three embryos were transferred into the uterine cavity on day 2 or 3 after oocyte retrieval. Clinical pregnancy was defined by the presence of a fetal sac at ultrasound examination 6 weeks after embryo transfer. For statistical evaluation (Wilcoxon test) each patient was used as her own control.

Results: In one case, stimulation had to be cancelled because of poor response. Five patients underwent IVF, two patients ICSI. Before and after sildenafil application, two or three embryos were transferred, in six cases with good quality, in one case with poor quality embryos. Two patients suffered from headache under sildenafil medication. In three cases, clinical pregnancy was confirmed by the presence of a fetal sac at ultrasound examination 6 weeks after embryo transfer. After sildenafil application the PI of the uterine arteries did not differ from the values before treatment (median: 2.35 versus 2.49; Wilcoxon test: $P = 0.80$). The PI of the ovarian arteries declined during treatment (median: 0.70 versus 0.79; Wilcoxon test: $P = 0.05$). On the other hand, the endometrial thickness was increased after application of sildenafil (median: 8.5 versus 6.3; Wilcoxon test: $P = 0.03$). Number of oocytes (median: $n = 7$ versus $n = 7$; Wilcoxon test: $P = 0.67$), fertilization rate (33 versus 60%; Wilcoxon test: $P = 0.15$) and embryo quality did not change during treatment.

Conclusion: Effects of sildenafil on perfusion of uterus and ovaries are controversial. Early reports on the benefit of sildenafil in assisted reproduction should be evaluated by placebo-controlled studies.

P-038. In the idiopathic infertility group of IVF patients a switch from T-cells to natural killer cells in follicular fluid is observed

Lukassen H.G.M.[1], Van der Meer A.[2], Lindeman E.[1], Wetzels A.[1], Wijnands F.[3], Van Lierop M.J.C.[3], Mosselman S.[3], Joosten I.[2] and Braat D.D.M.[1]

[1]Department of Obstetrics and Gynaecology and [2]Department of Bloodtransfusion and Transplantation Immunology, University Medical Center Nijmegen, Nijmegen and [3]Department of Pharmacology, NV Organon, Oss, The Netherlands

Introduction: Leukocytes and cytokines play a key role in ovarian physiology alongside gonadotrophins and traditional growth factors. Many reports describe cytokine levels in follicular fluid, but only a few studies focused on the cellular content. The question arises whether distribution of immune cell populations in follicular fluid varies depending on the cause of infertility and whether this influences the success rate of IVF treatment.

Materials and methods: We evaluated leukocyte subpopulations present in follicular fluid of patients undergoing IVF-embryo treatment treatment for idiopathic infertility in comparison to andrological infertility or tubal factor infertility (control group). Triple colour flow cytometry was used to discriminate T-cells and natural killer (NK) cell subsets.

Results: So far 121 follicles from 47 patients have been analysed. Only erythrocyte-contaminated follicular fluid ($n = 59$) from 36 patients contained sufficient leukocytes for analyses. The data show that the immune cell composition of follicular fluid differed significantly from that of peripheral blood leukocytes from the same patients. Follicular fluid from patients with idiopathic infertility ($n = 15$) contained a higher proportion of NK cells (19%), and a lower proportion of T cells (65%) as compared to the tubal factor or andrological infertility groups ($n = 21$), respectively 13% NK cells and 73% T-cells. These differences were statistically significant. In peripheral blood, no differences between these groups in the proportion of NK and T-cells were observed. Remarkably, in follicular fluid also the composition of the NK cell subset was different, with a relatively high percentage of immunoregulatory $CD16^{-}CD56^{++}$ NK cells.

Conclusion: These results show striking differences in distribution of lymphocyte subsets in follicular fluid between idiopathic infertility and other causes of infertility, possibly implicating an immunological basis for idiopathic infertility. The functional capacity of these cells with respect to fertilization rate, oocyte maturation and embryo quality warrants further investigation.

P-039. Fertilizing capability of frozen–thawed spermatozoa, recovered from microsurgical epididymal sperm aspiration and cryopreserved in oocyte-free human zona pellucida

Fusi F., Calzi F., Rabellotti E., Papaleo E., Gonfiantini C., Bonzi V., De Santis L. and Ferrari A.

Fisiopatology of Reproduction Unit, Ob/Gyn Department, San Raffaele Scientific Institute, University of Milan, Italy

Introduction: The possibility of achieving high fertilization rate with semen recovered by microsurgical epididymal aspiration (MESA) using intracytoplasmic sperm injection (ICSI) has introduced an effective treatment for obstructive azoospermic patients. The need to minimize surgical intervention to obtain viable spermatozoa after every failed cycle necessitates a more efficient method of sperm storage, even of a small number of spermatozoa, recruited in a single surgical aspiration. The aim of our study was to evaluate the efficacy of performing ICSI using spermatozoa stored and cryopreserved, either as single cells or as a small group, after injection into oocyte-free human zona pellucida (ZP).

Materials and methods: Spermatozoa were obtained from two severely azoospermic patients by MESA. The female partner underwent a long protocol of ovarian stimulation: 32–26 h after human chorionic gonadotrophin administration, oocytes were recovered by transvaginal ultrasound-guided needle aspiration. A total of 18 oocytes were recovered; of these, eleven metaphase II oocytes were injected using fresh spermatozoa simultaneously recovered from MESA. Ten embryos were obtained (fertilization rate pair to 90.9%); they all were transferred (five embryos per patient), and no pregnancy was documented. Empty ZP were recruited from the respective female partner oocyte not injected. Motile spermatozoa were injected into ZP (15–20 per ZP) using the intracytoplasmic injection needle and cryopreserved until the thawing procedure. More than 75% of recovered spermatozoa showed vitality after the retrieval from thawed ZP and were injected.

Results: Four cycles of ovulation induction produced a total of 35 oocytes (mean 8.75 oocyte/cycle), 30 of which were at metaphase II and injected using thawed spermatozoa. Fertilization was assessed in 17 of them (pair to 56.6% per injected mature oocytes) and cleavage was observed in 13 of 17 fertilized oocytes (pair to 76.4%). Embryo transfers were performed in all four cycles (mean 3.25 embryo/cycle). One singleton pregnancy was achieved.

Conclusions: Our preliminary data show that ICSI performed with frozen-thawed spermatozoa is able to achieve satisfactorily high fertilization rate

and embryo cleveage. Furthermore, oocyte free human zona pellucida storage allows cryopreservation spermatozoa from very poor specimens, as in MESA retrieval, representing an efficient alternative to repeated surgical procedure in severe azoospermic patients.

P-040. Stimulation with FSH alone or in combination with HMG does not effect the cryosurvival and cleavage rates of thawed human embryos

Karlikaya G., Yakin K., Kahraman S., Dönmez E., Cengiz S. and Vanlioglu F.

Istanbul Memorial Hospital, Reproductive Endocrinology and ART Unit, Istanbul, Turkey

Introduction: The purpose of the study was to compare the cryosurvival, cleavage, pregnancy and ongoing pregnancy rates for the groups in which ovarian stimulation was performed either with FSH alone or in combination with human menopausal gonadotrophin (HMG).

Materials and methods: A retrospective analysis was performed on the data obtained from the files of 74 women who had a frozen thawed embryo transfer. The female age, basal FSH concentrations, peak oestradiol concentration on the day of human chorionic gonadotrophin (HCG), total number of metaphase II (MII) oocytes retrieved, the number of frozen embryos, the phase of cryopreservation, the clinical protocol for endometrial preparation, number of embryos transferred, cryosurvival and cleavage rates, as well as the pregnancy results were recorded and compared between two groups.

Results: Ovarian stimulation was performed with the use of FSH alone in 55 cycles, and in combination with HMG in 19 cycles. Cryopreservation was performed at the 2-pronuclear stage, 2nd and 3rd day of cleavage for 37, 23 and 14 cases respectively. There was no statistically significant difference between the groups in terms of female age and basal FSH concentrations. The peak level of oestradiol on the day of HCG was significantly higher in cycles with HMG supplementation (2319 versus 4375 pg/ml) ($P < 0.05$). However, the mean number of MII oocytes and number of frozen embryos as well as the number of embryos transferred did not reveal a significant difference. Although the cryosurvival rate was higher in 'FSH alone' group, the difference lacked statistical significance (79.1 versus 68.5%) ($P = 0.07$). The cleavage rates were similar for both groups. The pregnancy rate was higher in the FSH + HMG group but the difference was not statistically significant (32.7 versus 47.4%). The results are summarized in the Table I.

Table I.

	FSH ($n = 55$)	FSH + LH ($n = 19$)	*P*
Age (years)	29.5 ± 4.4	28.7 ± 4.5	NS
Oestradiol (pg/ml)	2319 ± 959	4375 ± 863	< 0.05
Metaphase II oocytes	16.8 ± 5.5	16.3 ± 5.5	NS
Cryosurvival rate (%)	79.1	68.5	–0.07 (NS)
Cleavage rate (%)	88.9	86.7	NS
Embryos transferred	3.6 ± 1.2	3.5 ± 1.2	NS
Pregnancy rate (%)	32.7	47.4	NS

NS = not significant.

Conclusion: In macaque embryos, the cryosurvival and viability of thawed embryos were found to be greater when follicle development was achieved with FSH + LH versus FSH alone. This was not true for human embryos. We could not find any statistically significant difference between cryosurvival or cleavage rates between the groups that were stimulated either with FSH alone or in combination with HMG. Over supression of LH during ovarian stimulation was suggested to compromise the cytoplasmic maturity and subsequent development of the growing embryos. Addition of HMG into stimulation protocols might improve the developmental capacity of embryos to overcome any cryodamage. On the contrary to the findings in macaque embryos, the role of HMG supplementation in cryosurvival is questionable.

P-041. Comparison of unilateral aspiration with albumin therapy to prevent ovarian hyperstimulation syndrome in assisted reproduction cycles: a prospective randomized study

Madani T.[1] and Tehrani Nejad E.SH.[1,2]

[1]Department of Endocrinology and Infertility, Royan Institute, P.O.Box 19395-4644 and [2]Valie-Asr Hospital, Tehran Medical University, Tehran, Iran

Introduction: The prevention of severe ovarian hyperstimulation syndrome (OHSS) is one of the problems in assisted reproduction centres. The objective of this study was to compare the preventive effect of follicular aspiration and albumin therapy in OHSS in assisted reproduction cycles.

Materials and methods: The study included patients undergoing a long protocol for ovarian stimulation in IVF or intracytoplasmic sperm injection programme. The OHSS patients had oestradiol >3000 pg/ml on the day of HCG administration. The number of follicles by transvaginal sonography in each ovary was >20. One hundred and six patients at risk for development of severe OHSS according to the above criteria were divided into two groups. Group I: 57 patients who received albumin (50 g/500 ml normal saline) at the day of follicular puncture. Group II: 49 patients who had unilateral follicular aspiration before HCG (10 000 IU) administration.

Results: The number of oocytes retrieved in group I was more than in group II ($P < 0.05$). There was no difference in prevention of OHSS by two treatment methods (56/57, 48/49 respectively) and one patient in each group had clinical symptoms of severe hyperstimulation and was hospitalized.

Conclusions: These data indicated that albumin therapy or aspiration of follicles had the same effect on prevention of severe OHSS. But albumin therapy is preferable because aspiration is an invasive method and the number of retrieved oocytes in albumin therapy was higher than that achieved by aspiration of follicle.

P-042. Outcome after transfer of frozen embryos derived from ICSI or conventional IVF

Hammadieh N., Sharif K., Afnan M., Ola B., Papaioannou S. and Eissa M.

Assisted Conception Unit, Birmingham Women's Hospital, Edgbaston, Birmingham B15 2TG, UK

Introduction: Intracytoplasmic sperm injection (ICSI) has a better outcome in comparison with conventional IVF. The question is whether ICSI cryopreserved embryos will also have better outcome compared with cryopreserved embryos from IVF in frozen embryo transfer (FET) cycles. A few studies carried out so far have given conflicting results; in addition, the numbers of analysed cycles were small. This study was designed to compare the embryo survival, implantation, pregnancy, biochemical pregnancy, miscarriage and clinical pregnancy rates between frozen embryos derived from IVF and ICSI in a FET programme. This information is important for patient's counselling.

Materials and methods: For this analysis, we included all FET cycles in the Assisted Conception Unit between January 1998 and October 2000. A total of 634 cycles was analysed, 433 cycles were IVF and 201 cycles were ICSI. Mean female age (33.9 versus 32.3 years) and mean number of embryos transferred (2.5 versus 2.2 embryos), for IVF and ICSI respectively, were similar.

Results: There was no significant difference in outcome between FET cycles with cryopreserved embryos derived from IVF and those from ICSI. See Table I.

Table I. Embryo survival and pregnancy outcome after transfer of frozen-thawed embryos (in FET cycles) derived either from IVF or ICSI

Variable	Cryopreserved IVF	Cryopreserved ICSI
No. cycles with cryopreservation	433	201
No. embryos cryopreserved	3175	1386
No. embryos thawed	1602	661
No. embryos survived thaw (%)[a]	1235 (77.1)	533 (80.6)
% implantation rate (FH/ET)[a]	9.3	12.0
Pregnancy rate (positive HCG) (%)[a]	112 (26.5)	53 (26.8)
Biochemical pregnancy (pregnancy loss before FH detected) (%)[a]	21 (18.8)	7 (12.3)
Miscarriage (pregnancy loss after FH detected) (%)[b]	7 (6.3)	1 (1.9)
Clinical pregnancy (FH on ultrasound scan) (%)[a]	80 (18.5)	43 (21.5)

[a]Yates' corrected χ^2-test. [b]Fisher χ^2-test.
FH = fetal heart; ET = embryo transfer.

Conclusions: The outcome after transfer of cryopreserved embryos derived from ICSI is similar to that from cryopreserved embryos derived from conventional IVF, and patients should be counselled accordingly.

P-043. Influence of multiple transrectal electroejaculations on sperm parameters and ICSI outcome

Giulini S.[1], Pesce F.[2], Maxia N.[1], Marsella T.[1], Salvatori M.[1], Volpe A.[1] and Battaglia C.[1]

[1]Department of Obstetrics and Gynecology and [2]Department of Urology, University of Modena and Reggio Emilia, Modena, Italy

Introduction: One of the major consequences of spinal cord injury (SCI) is infertility due to anejaculation. Transrectal electroejaculation (TREE) is one of the most commonly used method to obtain spermatozoa from these patients. For various reasons, the quality of semen obtained is almost always poor and pregnancy rate was low until the advent of intracytoplasmic sperm injection (ICSI). The sperm parameters in SCI patients is similar to epididymal necrospermia (very low sperm motility and viability). In epididymal necrospermia, frequent ejaculations improve sperm motility and viability. The aim of this study was to compare sperm parameters and ICSI outcome in patients who underwent single or multiple electroejaculations (EE) prior to ICSI.

Materials and methods: Thirty-four patients were included with SCI as the exclusive factor of infertility. They were randomly assigned to a single (group I, $n = 18$) or to multiple (basal, after 1 and 3 months) EE (group II, $n = 16$) prior to ICSI. Exclusion criteria were: hormonal and/or testicular dysfunction, female infertility and female age >38 years. In all cycles, we analysed the sperm parameters (according to World Health Organization criteria) and the ICSI outcome with respect to pregnancy rate. The EE were obtained by a Seager rectal probe. The female partners underwent standard long protocol ovarian hyperstimulation.

Results: In group I, two patients from whom no ejaculate was obtained were excluded from the study and submitted to testicular sperm aspiration (TESA). The mean sperm volume was similar between the two groups (group I 1.4 ± 0.6 ml; group II basal 1.3 ± 0.8 ml, 1 month EE 1.4 ± 0.4 ml, 3 months EE 1.6 ± 0.5 ml). The rate of normal sperm morphology was not significantly different (group I 28%; group II basal 21%, 1 month EE 32%, 3 months EE 40%). After 1 month (27×10^6/ml) and 3 months (34×10^6/ml) EE sperm concentrations were significantly ($P < 0.001$) higher than the basal concentrations both in group I (18.7×10^6/ml) and group II (18×10^6/ml). The rate of total sperm motility increased significantly ($P < 0.001$) after 1 month (80%) and 3 months (90%) EE with respect to the basal values both in group I (40%) and group II (30%). This significant improvement was due to the increased rate of in-situ and undulatory sperm motility ($P < 0.001$) after 1 month (70%) and 3 months (75%) EE with respect to the basal values both in group I (35%) and group II (25%). The linear progressive motility did not change significantly. In all, couples achieved nine pregnancies (PR 28%): two singletons, five twins and two triplets. The PR/patient in group II was significantly higher than in group I (37.5 versus 18.5%, $P < 0.001$).

Conclusion: In patients with SCI, repetition of electroejaculations is a useful method of improving the sperm parameters and consequently to enhance pregnancy rate.

P-044. Artificial preparation of the endometrium with no previous gonadotrophin-releasing hormone agonist suppression

Borini A.[1], Dal Prato L.[1], Cattoli M.[1], Bianchi L.[1], Maccolini A.[1] and Flamigni C.[2]

[1]Tecnobios Procreazione, Centre for Reproductive Health, Bologna and [2]Department of Obstetrics and Gynaecology, University of Bologna, Bologna, Italy

Introduction: Endometrial preparation with exogenous steroids after pituitary down-regulation with a GnRH analogue allows planning the date of embryo thaw and transfer, reducing the risk of cycle cancellation. However, some disadvantages should be considered: the high cost of GnRH analogue, the risk of hypo-oestrogenic side-effects prior to hormonal replacement and the long time needed for preparation. The aim of this study was to verify the efficacy of endometrial preparation with transdermal oestradiol in two different regimens for frozen–thawed embryo transfer, without pre-treatment with GnRH agonist, in women with normal ovarian function.

Materials and methods: In all, 708 women were randomized in three treatment groups. In group 1 (116 patients) endometrial preparation began on day 1 of menstrual cycle with 17β-oestradiol transdermal patches in a steadily increasing dosage from 100 to 300 μg for at least 12 days. Patches were replaced every 84 h. In group 2 (150 patients) the starting dose was 200 μg oestradiol patches, increased to 300 μg after 7 days. As control group (group 3), in 146 patients depot GnRH-a was administered in the luteal phase before starting oestradiol as for group 1. The dose was increased to 400 μg in each group whenever the endometrial thickness was <8 mm. After ultrasound confirmation of endometrial thickness >8 mm and no ovarian activity, progesterone in oil (100 mg i.m. daily) was added. One to three embryos were transferred via transcervical route 48 h later. Hormonal treatment was continued at least until pregnancy test performed 15 days after transfer.

Results: Age (both at freezing and thawing times) and endometrial thickness (9.7 ± 1.4, 9.5±1.5 and 9.4 ± 1.4 mm) were similar in the three groups. Three cycles (2.6%) were cancelled in group 1, and six (4%) in group 2, for evidence of ovulation or luteinized endometrium. Embryo survival after freezing–thawing (80.1% in group 1, 76.6% in group 2 and 77.1% in group 3) and the numbers of embryos transferred per patient (2.1 ± 0.7, 2.1 ± 0.6 and 2.1 ± 0.7) were comparable in the three groups. No significant differences were seen between the three groups in terms of pregnancy (22.5, 24.1 and 19.7%), abortion (16, 11.7 and 17.8%) and implantation rates (11.4, 11.9 and 10.4%).

Conclusions: Endometrial preparation for frozen–thawed embryo transfer based exclusively on steroid administration appears to be as effective as the more conventional protocol involving preliminary desensitization with GnRHa, maintaining the same success rate. The procedure is simpler and cheaper and more convenient for both clinician and patient. Increasing the starting dose of transdermal oestradiol from 100 to 200 μg does not bring any significant advantage in terms of quality of endometrium and success rate. In any case, to minimize ovarian activity, it is important to start treatment in the very early follicular phase (day 1–2).

P-045. ICSI using testicular sperm reveal unexpected partial obstruction in severe oligozoospermic patients: prospective study

Kamal A., Mansour R.T., Fahmy I., Serour G. and Aboulghar M.

The Egyptian IVF-ET Center, Maadi, 11431, Cairo, Egypt

Introduction: Testicular sperm extraction (TESE) is mainly indicated for intracytoplasmic sperm injection (ICSI) in patients with azoospermia. In certain circumstances in oligozoospermic patients, not enough spermatozoa can be found to perform ICSI on the day of oocytes retrieval. Repeated trials using ejaculated available spermatozoa usually end in failure to conceive. We decided to perform ICSI in these cases using

testicular spermatozoa and to compare the outcome of ICSI + TESE done for these patients with ICSI cycles done previously using ejaculated spermatozoa for the same couples.

Materials and methods: Prospective study including 65 severe oligozoospermic patients. They underwent TESE, which were used for ICSI cycles. The outcomes of ICSI using testicular or ejaculated spermatozoa were compared with respect to fertilization (FR) and pregnancy (PR) rates.

Results: In 10 out of 65 (15.4%) patients, partial obstruction was diagnosed and the testis biopsy showed normal spermatogenesis. The fertilization and pregnancy rates were 55.4 and 28.5% respectively, compared with 49.5 and 0% in previous trial using ejaculated spermatozoa. The difference in the pregnancy rate between the two groups is statistically significant. In the rest (55 cases) the testis biopsy showed impaired spermatogenesis. The FR and PR were 42.49 and 21.3% respectively, which were similar to outcomes of ICSI using ejaculated spermatozoa (FR = 40%, PR = 15.6%) for the same patients in previous trials.

Conclusion: In patients with severe oligozoospermia, the diagnosis of partial obstruction should not be ignored. The present study demonstrates that 15% of severe oligozoospermic patients had normal spermatogenesis. The diagnosis of partial obstruction would be missed if testis biopsies were not done. We suggest performing TESE + ICSI in patients with severe oligozoospermia in cases with repeated failure using available ejaculated spermatozoa.

P-046. Sperm injection site at ICSI influences the embryonic development and pregnancy rates

Lee D.S., Yoon S.H., Lee S.W., Moon J.H., Son W.Y. and Lim J.H.

IVF Laboratory, Maria Women's Hospital, Seoul, Korea

Introduction: Fertilization, embryonic development and pregnancy might be affected by damaging the meiotic spindle as a spermatozoon is injected at the 3 o'clock position (with first polar body at 12 o'clock position) during intracytoplasmic sperm injection (ICSI). This study was carried out to investigate whether sperm injection performed to 4 o'clock position may improve the fertilization, embryonic development and pregnancy rates compared to 3 o'clock position at ICSI.

Materials and methods: In experiment 1, 192 mature human oocytes were collected from 20 of patients undergoing ICSI. The collected sibling oocytes were divided into two groups: group 1 (n = 96) in which a spermatozoon was injected to the 3 o'clock position with polar body at 12 o'clock position; group 2 (n = 96) in which a spermatozoon was injected at the 4 o'clock position with polar body at 12 o'clock position. In experiment 2, 114 patients undergoing ICSI were randomly divided into two groups: group 1 (n = 66 patients), in which sperm injection was performed at the traditional 3 o'clock position (no. of oocytes = 607), and group 2 (n = 48 patients) which sperm injection was performed at the 4 o'clock position (no. of oocytes = 421). Normal fertilization was defined by the presence of two pronuclei at 17–19 h post ICSI, and embryonic development was assessed by grading developmental morphology on the third day of culture. We compared the clinical outcomes between both sperm injection sites in each experiment.

Results: The normal fertilization rate of the sibling oocytes which injected at the 4 o'clock position (site II) was significantly higher than that at the 3 o'clock position (site I) (83.0 and 71.9% respectively, $P < 0.05$). No difference was observed between site I and site II in terms of the cleavage rates of the sibling oocytes (98.6 and 96.3% respectively). However, significantly more embryos with ⩾4-cell and good grade on day 2 were obtained from sibling oocytes injected at site II (49.4 and 46.8% respectively) than at site I (38.2 and 41.2%, respectively) ($P < 0.01$). More embryos transferable on day 3 were derived from injection at site II than at site I (44.2 and 27.9% respectively, $P < 0.01$). Significantly higher clinical pregnancy rate was obtained following injection at site II than at site I (56.3 and 30.2% respectively, $P < 0.01$), and implantation rate was also significantly higher for injection performed at site II than at site I (27.7 and 14.3% respectively, $P < 0.01$).

Conclusions: The present study indicates that a spermatozoon should be injected at the 4 o'clock position with first polar body at 12 o'clock position to avoid the metaphase II spindle area and to obtain higher embryonic development, implantation and pregnancy rates at ICSI.

P-047. Laser-assisted ICSI: a new approach to obtain higher oocyte survival and embryo quality rates

Abdelmassih O.S., Abdelmassih V., Abdelmassih R. and Nagy P.Z.

Clínica e Centro de Pesquisa e Reprodução Humana Roger Abdelmassih, Rua Maestro Elias Lobo, 805, 01433-000 São Paulo, São Paulo, Brazil

Introduction: It is a general experience that intracytoplasmic sperm injection (ICSI) procedure is associated with degeneration of some of the microinjected oocytes. This is partly due to technical conditions that can be improved; however, degeneration of oocytes occurs even when maximum care is exercised, especially when the oolemma is very fragile resulting in a sudden breakage of the membrane during ICSI. This probably does not affect the results of most ICSI cycles, but embryo transfer for patients, especially those with very few oocytes, might have to be cancelled occasionally. In order to be able to minimize the risk associated with microinjection we applied a new method: drilling a micro-hole on the zona pellucida on the oocytes by laser beam just prior to ICSI that permits the penetration of the microneedle without any trauma. The efficacy of this new technique was analysed on sibling oocytes in a prospective design.

Materials and methods: A total of 32 ICSI treatment cycles were included in the study was performed between August and December of 2000. Patients were selected for the study on the bases that they had one or more previous ICSI cycles with high degeneration rate of oocytes following microinjection. Patients underwent ovarian stimulation using recombinant FSH after pituitary down-regulation with a long leuprolide acetate protocol. Oocytes from the same patients were divided into two groups. In group 1, oocytes were submitted to laser-assisted ICSI (LA-ICSI) using a 1.48 μm diode laser (Fertilase) to drill the zona pellucida just prior to microinjection. Using three to five low energy pulses a channel with small diameter (5-6 μm) was drilled. The injection pipette was introduced through this channel and ICSI was performed as usual. In group 2, oocytes were submitted to the conventional ICSI (C-ICSI) procedure. One-way analysis of variance, Kruskal-Wallis and χ^2-tests were applied as appropriate.

Results: The mean age (± SD) of the women in these 32 cycles was 32.7 years (± 4.3). A total of 415 oocytes were recovered and 338 of them were at the metaphase II stage (81.4%). Data on the 32 cycles comparing LA-ICSI versus CI are presented in Table I.

Table I. ICSI outcomes

	Laser-assisted	Conventional	P
No. of injected oocytes	201	137	
Normal fertilization (%)	154 (76.6)	94 (68.6)	< 0.0001
Oocytes survived (%)	153 (99.6)	72 (84)	< 0.0001
Embryos with >6 cells on day 3 (%)	118 (76.5)	54 (57.3)	0.0024
Good quality embryos (%)	95 (61.6)	43 (45.3)	0.0239

Conclusions: The results of this study show that creating a micro-hole on the zona pellucida of the oocyte by laser beam prior to ICSI assists a less traumatic penetration of the injection needle into the ooplasm. The degeneration rate of oocytes is minimal and consequently the survival rate is very high. Developmental speed and morphological quality of embryos is better after laser-assisted ICSI than after conventional ICSI. This improvement in cleavage and quality of embryos may be related to the fact that oocytes in the LA-ICSI group were not compressed at all during microinjection that probably resulted in a better preserved microfilament structure to effect cell division. We suggest the use of laser-assisted ICSI in all cases that are associated with difficult zona pellucida penetration and/or with fragile oolemma or where patients have very few oocytes available.

P-048. Hydroxyethylstarch versus human albumin for treatment of severe ovarian hyperstimulation syndrome

Abramov Y., Fatum M., Fasouliotis S.J., Abrahamov D. and Schenker J.G.

Department of Obstetrics and Gynecology, Hadassah Hebrew University Medical Center, Ein-Kerem, Jerusalem, Israel

Introduction: At present, human albumin is the most widely used colloid solution for treatment of severe ovarian hyperstimulation syndrome (OHSS). Yet recent reports have doubted its effectiveness in the light of its short biological half-life and low molecular weight (40 kDa), as well as its tendency to escape through hyperpermeable capillaries. Hydroxyethylstarch (HES) 6% is a powerful, high molecular weight (200–1000 kDa) colloid solution with longer biological half-life, which serves for treatment of severe intravascular volume depletion states such as burns, haemorrhagic and septic shock. This clinical study compares the efficacy and safety of both solutions in treating severe OHSS.

Materials and methods: This controlled cohort study included 16 IVF patients with severe OHSS. Six patients received HES 6% alone as a colloid solution (group I), while 10 others received only human albumin (group II). We examined the patients' clinical course, hospitalization period, number of paracenteses and complications, as well as conception rates and perinatal outcome.

Results: There were no significant differences between the two study groups in terms of patient's age, fertility treatment and severity of OHSS. Both groups received similar volumes of oral and intravenous fluids. However, daily urine output during the first 5 days of hospitalization was higher in group I (3582 ± 1780 versus 2557 ± 1032 ml), and fewer patients from this group required abdominal (33 versus 80%) and/or pleural (0 versus 20%) paracenteses. Mean hospital stay was about 3.3 days shorter (15.7 ± 5.7 versus 19 ± 8.2 days) in group I. No thromboembolic phenomena or solution-related side-effects occurred in either group. Conception (67 versus 70%) and miscarriage (17 versus 28%) rates were not significantly different, and no fetuses with congenital malformations were detected in either group.

Conclusions: Hydroxyethylstarch 6% seems to be more effective than human albumin in maintaining urine output, reducing number of abdominal paracenteses, and shortening hospitalization period in severe OHSS.

P-049. A European multicentre prospective randomized study to assess the use of assisted hatching in different patient populations

Primi M.-P.[1], Senn A.[1], Pannatier A.[1], Rink K.[2], Delacrétaz G.[2], Montag M.[3], van der Ven H.[3], Mandelbaum J.[4], Veiga A.[5], Barri P.[5] and Germond M.[1]

[1]Reproductive Medicine Unit, Department of Gynaecol.-Obstet., CHUV, CH-1011 Lausanne, Switzerland, [2]Institut d'Optique Appliquée, EPFL, CH-1015 Ecublens, Switzerland, [3]Department of Gynaecol.-Obstet., University of Bonn, D-53105 Bonn, Germany, [4]IVF and Reproductive Biology Laboratory, Necker Hospital, F-75015 Paris, France and [5]Dexeus Institute, E-08017 Barcelona, Spain

Introduction: Several clinical studies using various assisted hatching (AH) techniques have investigated the effect of AH on embryo implantation. All retrospective studies conclude that AH enhances both the implantation (IR) and clinical pregnancy (CPR) rates in various patient populations. The randomized studies performed in unselected patients appear to show that IR and CPR are not augmented by AH. Nevertheless, in some sub-groups of poor prognosis patients, i.e. with advanced maternal age, elevated basal FSH, repeated IVF failures, thick zona pellucida (ZP) or frozen-thawed embryo transfers, AH is often associated with higher IR and CPR. A prospective randomized study was performed in four European university centres equipped with the same laser system. The main objectives were to assess the benefit of AH in four groups of patients and the efficacy of an associated immunosuppressive/antibiotic therapy. Secondary objectives were to establish the roles of the morphological characteristics of the transferred embryos, the ZP thickness and the opening diameter on the IR and to initiate a follow-up of the pregnancies up to delivery.

Materials and methods: A total of 377 patients, recruited from January 1997 to December 1999, were allocated in one of the following four groups depending on their specific AH indications. Group 1: patients who initiated their first transfer cycle of cryopreserved-thawed embryos; group 2: patients who had experienced two previous nidation failures of cryopreserved-thawed embryos and initiated their third transfer cycle; group 3: patients who initiated a first transfer of fresh embryos with a poor prognosis of conception (basal FSH >10 IU/l and/or age >37 years); group 4: patients who initiated a fourth transfer cycle after three previous implantation failures of fresh embryos (involving the transfer of at least six good quality embryos). ZP was microdrilled using a 1.48 µm diode laser (Fertilase®; Medical Technologies Montreux, Switzerland). In groups 1 and 3, microdrilling was performed or not (controls), according to a randomization list, whereas in groups 2 and 4 all embryos were microdrilled. The patients receiving laser-drilled embryos were further randomized in a double-blind trial, testing the role of a placebo/immunosuppressive + antibiotic treatment (IA) during the preimplantation period (2 days prior up to 5 days after embryo transfer). Clinical pregnancies were diagnosed by the presence of fetal sacs with cardiac activities.

Results: In group 1, a significantly lower CPR was observed after AH without IA (1/62, 1.6%) compared to AH with IA (6/56, 10.7%; $P < 0.02$) and to control (8/53, 15.1%; $P < 0.02$). A similar trend was observed in group 3 in which these rates were changed to 9.5% (2/21), 21.7% (5/23; NS) and 23.8% (5/21; NS) respectively. In group 2, AH led to comparable CPR among patients receiving (3/39, 7.7%) or not (4/39, 10.3%) IA therapy. Similar observations were made in group 4 for AH/with IA (5/35, 14.3%) and AH/without IA (5/30, 16.7%). Data obtained on ZP thickness and embryo morphology will be evaluated and discussed.

Conclusions: Recruitment of the patients appeared much more difficult than initially anticipated. The study had thus to be ended before the expected number of patients ($n = 600$) was reached. Despite the premature ending of the study, the data indicate that AH does not lead to higher clinical pregnancy rates in older patients (group 3) and in patients initiating their first cryo cycle (group 1). In these two groups, AH treatment appears to have a negative effect when not supported by IA therapy. When AH is performed after repeated implantation failures (groups 2 or 4), the pregnancy rates are not significantly improved by the IA treatment and they remain below those expected.

P-050. The utilization of a group culture system to increase blastocyst viability

Egashira A., Motoishi M., Sugioka M., Umebayashi T., Fukuda K., Higashizima T. and Kuramoto T.

Kuramoto Women's Clinic, 4-8-8F, Hakataeki-Chuogai, Hakata-ku, Fukuoka, Japan

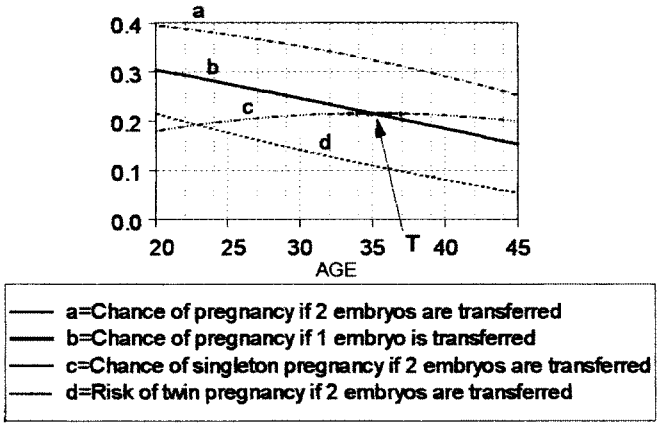
Introduction: Recently, sequential media have been developed to support preimplantation human embryo development up to blastocyst stage. Thus, blastocyst transfer increased the implantation rates. In this study, to determine whether the group culture system of human embryos from day 3 improves the developmental ability to the blastocyst stage, we compared the rates of blastocyst formation and quality of morphological graded blastocysts on day 5 between the group culture system and the single culture system.

Materials and methods: Zygotes after insemination or intracytoplasmic sperm injection were transferred individually into a 20 µl drop of culture medium in a culture dish covered with mineral oil. The dish was placed in an incubator (37°C, 5% CO_2, 5% O_2 and 90% N_2) until day 3. On day 3 morning, 5-8-cell embryos with <30% fragmentation were divided into three groups randomly. Single culture included one embryo (group 1) and group culture included two embryos (group 2) or five embryos (group 3). The embryos were transferred into a 20 µl drop of fresh culture

medium in a culture dish covered with mineral oil and cultured until day 5 under the same conditions as before day 3.

Results: On day 4, no significant differences in morular formation rates could be found between the three groups. However, on day 5 morning, blastocyst formation rates in group 2 and group 3 had increased to 60 and 62% respectively, compared with 47% in group 1. In addition, there was no difference in the number of blastocysts with good or fair grade morphology between group 1 (39%) and group 2 (50%), but the number of blastocysts with good or fair grade morphology significantly increased in group 3 (76%).

Conclusions: These results indicate that a group culture system of human embryos from day 3 improved the rates of blastocyst formation and blastocyst quality. The improvement of blastocyst formation and quality in a group culture system may be due to the embryo interaction such as autocrine and/or paracrine. Therefore, by utilizing a group culture system it should be possible to select a single viable blastocyst and decrease the risk of multiple pregnancy.



Conclusion: Application of this model enables the reduction of twin pregnancy rate without compromising singleton pregnancy rate.

P-051. Selecting IVF patients for single embryo transfer: a prediction model

Hunault C.[1], Eijkemans M.J.C.[1], Pieters M.H.E.C.[2], Fauser B.C.J.M.[2], Habbema J.D.F.[1], Macklon N.S.[2]

[1]Department of Public Health, EMCR, Rotterdam, The Netherlands [2]Department of Obstetrics and Gynaecology, EMCR, Rotterdam, The Netherlands PO box 1738, 3000 DR Rotterdam, The Netherlands, E-mail: hunault@mgz.fgg.eur.nl

Introduction: Multiple pregnancies following IVF remain a major problem with IVF. In order to reduce their number, and the associated perinatal mortality and morbidity, many centres have adopted a policy of two-embryo transfer. Twin pregnancy rates remain high nevertheless. To identify which patients would benefit most from single embryo transfer (SET), the individual probabilities of pregnancy and twin-pregnancy following transfer of one and two embryos are required. We constructed a prediction model to enable selection of patients for elective SET.

Material and methods: We analysed 642 first cycles, with transfer of two embryos, on day 3, 4 or 5 after follicular aspiration. Cycles with donor oocytes, cryo-thaw embryo and intra-cytoplasmic sperm injection were excluded. Because the implantation rates of embryos transferred in the same uterus are not independent, we used a published method to predict the outcome following SET from data relating to two embryo transfer cycles. This method assumes that survival of an embryo transferred to the uterus depends on its own inherent viability and the receptivity of the uterus that it shares with the other transferred embryo. We entered the patient's specific variables as potential factors affecting the uterine receptivity and woman- and embryo-specific variables as potential factors affecting embryo viability. Selection of the variables was done using univariate and then stepwise multivariable regression analyses. The model best fitting to the data was chosen.

Results: Among the 642 women, 170 achieved an ongoing pregnancy of which 60 were multiple pregnancies. We identified five predictors of the embryo viability: the woman's age, the number of oocytes obtained, the embryo development stage score and the morphology score of the transferred embryo, and the day of embryo transfer. The uterine receptivity was not statistically influenced by patient's specific covariates. Given the woman's age, the probabilities of pregnancy and twin-pregnancy after transfer of one or two top quality embryos are shown in Figure 1. The figure shows these probabilities for two top quality embryos transferred on day 3. The benefit of SET is the reduction in the twin-pregnancy rate (line d), and the drawback of SET the reduction of pregnancy rate (difference between curves a and b). Under the threshold age T, the chance of pregnancy if one embryo is transferred is higher than the chance of singleton pregnancy if two embryos are transferred.

P-052. Cryopreservation of different stages of human blastocysts obtained by sequential culture protocols

Van den Abbeel E., Verheyen G., Joris H., Camus M., Devroey P. and Van Steirteghem A.

Centre for Reproductive Medicine, Dutch-speaking Brussels Free University, Laarbeeklaan 101, 1090 Brussels, Belgium

Introduction: The aim of the present study was to control the freezability of different stages of sequentially cultured human blastocysts.

Materials and methods: After replacement of two to three good quality embryos on day 2 of the oocyte collection cycle (OCC), all supernumerary embryos were cultured up to the blastocyst stage in commercially available sequential culture media. Several types of blastocyst were frozen: type 1 and 2 blastocysts (cavitating embryos after compaction); type 3 blastocysts (expanded blastocysts); type 4 blastocysts (fully expanded blastocysts); and type 5 blastocysts (hatching blastocysts). They were frozen using a published freezing protocol. The cryoprotectant was removed in two steps (10 min in a 0.5 mol/l and 10 min in a 0.2 mol/l sucrose solution). After thawing and dilution the embryos were allowed to expand or re-expand by culturing them further for 24 h. The viability of frozen-thawed blastocysts was evaluated as follows: (i) immediate morphological survival (>50% of cells surviving) after thawing and dilution; (ii) expansion and (or) re-expansion of the blastocoelic cavity; (iii) implantation capacity of replaced surviving blastocysts.

Results: Freezing and thawing of human blastocysts was evaluated from August 1999 up to December 2000. We evaluated 1017 freezing cycles and cryopreserved 3129 blastocysts. We performed 364 thawing cycles and thawed 1116 blastocysts. The immediate morphological survival of blastocysts after thawing and dilution was 67% and after further culture 63% of surviving blastocysts expanded or re-expanded. In 280 replacement cycles (77% of thawing cycles) we replaced 564 blastocysts (51% of thawed blastocysts). Human chorionic gonadotrophin (HCG) measurement was positive in 54 replacement cycles (19% per replacement). Eleven of these ended in biochemical pregnancies (20% of positive HCG measurements) and seven ended in early miscarriages (13% of positive HCG measurements). We obtained 36 clinical pregnancies (13% clinical pregnancy rate per transfer) and diagnosed 36 clinical implantations (6.5% clinical implantation rate per blastocyst transferred). There were no differences in survival and expansion rates between the different stages of human cryopreserved blastocysts or between embryos originating from IVF cycles and from IVF cycles in association with ICSI.

Conclusion: Human blastocysts obtained by sequential culture protocols can be successfully cryopreserved and the results are not dependent on the pre-freezing developmental stage of human blastocysts or on the treatment procedure during the OCC. The outcome of cryopreservation, however, was considerably poorer than published outcome data on co-cultured blastocysts.

P-053. Higher rates of recovery with Puresperm density gradient compared to ISolate

Agarwal A. and Ranganathan P.

Center for Advanced Research in Human Reproduction and Infertility, Urological Institute and Department of Gynecology and Obstetrics, Cleveland Clinic Foundation, Cleveland, Ohio, USA

Introduction: The ideal semen processing technique should result in the selective recovery of good quality motile spermatozoa while maintaining a high total yield of recovered spermatozoa. It has been demonstrated that the use of density gradient media for the isolation of highly motile spermatozoa gives improved fertilizing ability and longevity in assisted reproductive procedures. The objective of our study was to test the efficacy of two commercially available density gradient media (Puresperm: a newly introduced product, Nidacon International, Gothenburg, Sweden; and ISolate, Irvine Scientific, Santa Ana, CA, USA) used for assisted reproduction. These two gradients are HEPES-buffered, isotonic, colloidal silica suspensions in two densities. Puresperm gradient: 40–80%; ISolate gradient: 50–90%.

Material and methods: We compared the pre- and post-wash sperm characteristics [count, motility, recovery rate, morphology, and reactive oxygen species (ROS) levels] of the sperm fractions obtained after processing 13 semen specimens with the two media. The above sperm parameters were further analysed in specimens categorized by normal motility ($\geqslant$50%, $n = 7$) or abnormal motility ($<$50%, $n = 6$) in undiluted semen.

Results: Semen specimens processed by Puresperm gave higher recovery of total sperm count ($P = 0.001$), and total motile sperm count ($P = 0.002$) than ISolate. The recovery rate for Puresperm was 37% higher than ISolate (mean $\pm$ SD 37.1 $\pm$ 22.1 versus 23.5 $\pm$ 14.5, $P = 0.006$). Motility and ROS levels were similar in the two fractions ($P = 0.96$ and 0.38, respectively). ROS production was insignificant in the two mature sperm fractions recovered after a 20 min centrifugation step involved in the gradient separation, indicating a lack of oxidative stress. Significantly higher rates of recovery were seen in Puresperm-processed specimens compared with ISolate in the normal motility group (47 $\pm$ 24.8 versus 28.5 $\pm$ 16.7, $P = 0.04$) and the abnormal motility group (25.6 $\pm$ 11.8 versus 17.8 $\pm$ 9.9, $P = 0.003$). Sperm fractions from both gradients showed a high percentage of normal sperm forms by both World Health Organization and Kruger's criteria.

Conclusions: We recommend the use of Puresperm for assisted reproduction purposes as higher rates of recovery of mature motile sperm in specimens processed for assisted reproduction is associated with higher fertilization and pregnancy rates. Puresperm not only gives higher recovery but is also cost-effective (ISolate is 42% more expensive than Puresperm in the USA).

P-054. Assisted reproduction in Germany: results of the German IVF registry based on 112 056 treatment cycles documented in 1998 and 1999

Felberbaum R., Dahncke W., Knuth U., Oppermann E., Van der Ven H., Blumenauer V., Bühler K., Fiedler K., Sterzik K. and Strowitzki T.

Deutsches IVF-Register, Bundesgeschäftsstelle Ärztekammer Schleswig-Holstein, Bad Segeberg, Germany

Introduction: German IVF groups have been voluntarily participating in a national IVF registry since 1982, and in 1999, 93 centres sent their computerized files to this registry. Since 1997 a software based on 'file-maker-pro' has been used nationwide by all centres participating in the Deutsches IVF Register (DIR).

Materials and methods: The catalogue of items covers all aspects of IVF treatment which can be completed in a retrospective or prospective manner. While for the overall data analysis only prospectively documented cycles were included, the calculations for all participating centres each were done on the basis of all documented cycles.

Results: Overall in 1999, 64 617 treatment cycles were reported to the German IVF registry, 58 817 of them being completely documented. Of these, 51 111 treatment cyles (86.9%) had been documented in a prospective manner. A total of 21 468 stimulation cycles for IVF and 20 658 for intracytoplasmic sperm injection (ICSI) produced a mean clinical pregnancy rate per embryo transfer of 24.3 and 24.7% respectively, showing an important decline of success rates when women were over 39 years old. A number of treatment cycles (2844) for the replacement of cryopreserved pronuclear (PN) stage oocytes revealed a clinical pregnancy rate per embryo transfer of 13.3%. Based on 47 439 treatment cycles documented in 1998, the take home baby rate (deliveries per treatment cycles) for IVF and for ICSI was 13.6 and 15.1% respectively, while cryopreservation of PN stage oocytes yielded a take home baby rate of 9.4%. The malformation rate for IVF or ICSI was 1.0 and 1.2% respectively. Data on 12 484 children born after IVF or ICSI treatments performed in 1998 and 1999 could be collected so far, reducing the lost for follow up rate to ~14%. No differences could be seen regarding gestational age and birth weight after IVF or ICSI treatment. 40% of these children born were multiples. There was a clear correlation between the number of embryos transferred and the incidence of twins and triplets. On the other hand the replacement of three embryos led to a mean pregnancy rate of 27.8% per embryo transfer in 1999, whereas replacing only two or one embryos resulted in pregnancy rate per embryo transfer of only 23.1 and 8.8% respectively.

Conclusions: The German IVF registry has become a well-established tool of quality control within reproductive medicine in Germany. Based on a nationwide prospective documentation and collection of data, the German IVF registry allows the establishment of a German quality standard, taking into account the special conditions of the German embryo protection law. The enormous effort of all participating centres must be gratefully acknowledged. The German IVF registry allows us to highlight specific problems arising from these legal conditions, e.g. the prohibition of any embryo selection while allowing the replacement of three embryos per transfer. These data will become very important within the political discussion on the legal regulations for assisted reproduction in Germany.

P-055. A change in embryo transfer policy based on female age to reduce triplet pregnancies in an IVF/ICSI programme.

Cowan L., Neal M.S., Gunby J., Ward S., Saunders P., and Daya S.

Hamilton Health Sciences Corporation, Hamilton, Canada.

Introduction: The concern about the exponential increase in multiple pregnancies has resulted in the recommendation that the number of embryos being transferred be limited to one or two. The purpose of this study was to evaluate the clinical outcomes of couples undergoing IVF/ICSI treatment and to determine whether a change in embryo transfer policy would be effective in reducing the number of triplet gestations, without compromising pregnancy potential.

Materials and methods: In our centre, a maximum of three day 3 embryos had been transferred to all women regardless of age or embryo quality, but from January 31st 2000 our policy was revised. Women $<$ 34 years old would receive a maximum of two embryos and women $\geqslant$ 34 years old would receive a maximum of three embryos. The new policy was based solely on the woman's age at the time of embryo transfer. The policy would be strictly adhered to, regardless of ovarian response and embryo quality. As usual, all women would be informed of the potential risks of multiple pregnancy and women $\geqslant$ 34 years of age would be given the option to have fewer embryos transferred, if desired. We evaluated the differences in number of embryos transferred, pregnancy rate, and triplet pregnancy rate prior to and after our policy change. The women underwent ovarian stimulation with leuprolide acetate in the long luteal protocol and recombinant FSH for IVF or ICSI treatment. Embryo selection for transfer was done at 72 hours, based on cell number and degree of cellular fragmentation. Supernumerary embryos were cryopreserved.

Results: The clinical outcomes of 292 fresh embryo transfers performed between April 1999 and December 2000 were analysed. Results are presented in the table below. With the revised embryo transfer policy, the mean number of embryos transferred decreased for women $<$ 34 years of age. Although women $\geqslant$ 34 years were given the choice to

transfer 2 embryos, there was no change in the mean number of embryos transferred in this group. There was a clinically important although not statistically significant ($P = 0.12$) decrease in the pregnancy rate for the < 34 years group after the policy revision, but no change for the $\geqslant 34$ years group. Surprisingly, a significant decrease was observed in the mean implantation rate in the < 34 years group, but not in the $\geqslant 34$ years group. A significant decline in the triplet rate (per clinical intrauterine pregnancy) was noted in the < 34 years group.

Table I.

Policy	# Cases	Mean (SD) Female Age (yrs)	Mean (Median) #Embryos Transferred	Pregnancy Rate % (n)	Mean Implantation Rate %	Triplet Rate % (n)
Old Policy						
< 34 yrs	45	30.8 (1.8)	2.47 ** (2.0)	57.8 (26/45)	38.5 ***	20.0 * (5/25)
>/= 34yrs	86	37.1 (2.2)	2.50 (3.0)	31.4 (27/86)	18.4	11.5 (3/26)
New Policy						
< 34yrs	59	30.9 (1.8)	1.93 ** (2.0)	42.4 (25/59)	28.0 ***	0 * (0/25)
>/= 34 yrs	102	37.3 (2.4)	2.49 (3.0)	34.3 (35/102)	18.8	2.9 (1/34)

Mean (+/- SD) *$P = 0.05$ ** $P = <0.001$ *** $P = 0.001$

Conclusion: Decreasing the triplet rate in an assisted reproduction programme can be achieved easily by decreasing the number of embryos transferred. However, pregnancy rate may be negatively affected when the decision to transfer two embryos is based solely on female age, without considering other factors, such as ovarian response, embryo quality and prior fecundity.

P-056. A prospective randomized controlled trial of Wallace and Rocket embryo transfer catheters in an IVF-embryo transfer programme

Lavery S.A., Ravhon A., Skull J., Ellenbogen A., Taylor J., Dawson K., Trew G., Margara R. and Winston R.

IVF Unit, Hammersmith Hospital, Du Cane Rd, London W12 0HS, UK

Introduction: Embryo transfer remains a crucial step in IVF. Ovarian stimulation, oocyte retrieval, fertilization and embryo culture have been extensively studied and are performed under tight control, while embryo transfer remains relatively unexplored. Attempts are constantly being made to improve catheter technology in an effort to improve success rates. The issue of catheter selection remains controversial with choice often programme-dependent. We wished to evaluate prospectively the performance of two different transfer catheters: Wallace (Sims Portex Ltd UK) and Embryon (Rocket Medical PLC UK).

Materials and methods: Over a period of 8 months, 308 patients were recruited and randomized: 160 to the Wallace and 148 to the Rocket group. Details describing the number of embryos transferred, the number of embryos available, physician factor, difficulty of transfer, visibility of catheter on ultrasound, patient discomfort (using a visual analogue score), day of transfer, implantation rate, clinical pregnancy rate, and cost of catheter were collected. Results were analysed on an intention-to-treat basis.

Results: The pregnancy rate in the Wallace group was 23.13% and in the Rocket group 22.97% ($P > 0.5$). The implantation rates were 13.2 and 11.8% respectively. The number of embryos transferred per number available, difficulty of transfer, change of catheter and patient discomfort were similar in both groups. Embryos were retained in the Wallace catheter in 22 cases and the Rocket catheter in four cases ($P < 0.05$). The catheter tip was clearly seen on ultrasound in 87.1% of transfers by the Rocket catheter and 76.9% of Wallace transfers ($P = 0.02$).

Conclusion: Pregnancy and implantation rates were similar when Wallace or Rocket catheters were used. The Rocket catheter was better seen under ultrasound and had a lower rate of retained embryos in the catheter after transfer. A change of catheter was required in 4.4% of the Wallace group and 4.1% of the Rocket group. Experience with different transfer catheters is recommended for difficult cases. When implantation and pregnancy results are equivalent, cost benefit may become the most influential factor in catheter selection.

P-057. Seminal quality of postpubertal boars exposed to 0, 12 and 24 h lighting

Sancho S., Pinart E., Garcia-Gil N., Badia E., Bassols J., Briz M. and Bonet S.

Unit of Porcine Assisted Reproduction, University of Girona, Campus de Montilivi s/n, 17071 Girona, Spain

Introduction: The semen quality of postpubertal boars is affected by several factors, such as temperature, light, diet, semen collection rhythm, age, hormones, illness, weight. However, there are conflicting views about the significance of these factors on seminal parameters.

Materials and methods: In order to determine the effects of light on seminal features, 30 postpubertal healthy boars of 8 months of age without significant differences among them in semen quality were randomly distributed in three groups: 10 males were assigned to 12 h lighting treatment (control group), 10 males to 24 h lighting treatment, and 10 males to 0 h lighting treatment. The duration of treatments was 2 months. In all groups the boars were kept at a temperature of 20 ± 1°C, fed with a nutritious diet, and subjected to a semen collection rhythm of twice per week. Semen samples used for analysis came from the last week of treatment. For each semen sample the parameters measured were: ejaculate volume and pH, sperm concentration, vitality and motility, and acrosomic resistance; the sperm morphology was assessed from the frequency of mature, immature, and aberrant spermatozoa. Statistical comparisons were made using the Student's *t*-test, at a significance level of $P < 0.05$. The volume and pH of the ejaculate were used as markers of the functional status of accessory glands, and the sperm concentration was an indicator of the testicular activity. The sperm vitality, sperm motility, and acrosomic resistance were indicators of the differentiation degree of the nucleus, flagellum, and acrosome respectively; alterations in these structures reflected disturbances in the testicular and/or epididymal activity. Immature spermatozoa were considered markers of impaired maturation along the epididymis, and aberrant spermatozoa were markers of defective testicular differentiation (primary anomalies) and epididymal differentiation (secondary anomalies).

Results. See Table I.

Table I.

Seminal parameters	Lighting treatment		
	12 h (controls)	24 h	0 h
Volume (ml)	176.3 ± 43.0	217.7 ± 53.5	184.4 ± 23.7
pH	7.34 ± 0.15	7.27 ± 0.1	7.24 ± 0.1
Sperm concentration (no./mm^3)	764 000 ± 231 000	205 000 ± 61 000[a]	227 000 ± 50 300[a]
Sperm motility (% motile)	98.8 ± 0.3	98.1 ± 0.8	96.8 ± 1.0
Sperm vitality (% live)	91.6 ± 2.2	90.2 ± 6.6	94.1 ± 2.3
Acrosomic resistance (% resistant)			
ORT 300 mOsmol	95.7 ± 1.5	96.2 ± 1.9	95.6 ± 1.6
ORT 150 mOsmol	64.0 ± 10.5	65.4 ± 12.1	53.5 ± 6.8
Sperm morphology (%)			
Mature spermatozoa	86.7 ± 1.5	82.9 ± 6.4	81.3 ± 5.3
Immature spermatozoa	8.0 ± 1.2	10.0 ± 1.4[a]	9.6 ± 1.3[a]
Aberrant spermatozoa	5.3 ± 0.8	7.1 ± 0.6	9.1 ± 4.1[a]
Head anomalies			
Primary	1.1 ± 0.3	1.5 ± 1.6	2.0 ± 0.4
Secondary	0.3 ± 0.2	0.3 ± 0.1	0.3 ± 0.1
Tail anomalies			
Primary	0.9 ± 0.3	1.2 ± 0.4	0.7 ± 0.2
Secondary	3.0 ± 0.6	2.5 ± 2.7	6.1 ± 3.7[a,b]

[a]Values significantly different from control treatment.
[b]Significant differences between 24 h and 0 h lighting treatments.

Conclusions: The data obtained in the present study indicated that 2 months of excessive or lack of light exposure produced subfertility in

the postpubertal boars as a result of a significant decrease in the sperm concentration, whereas the volume and pH of the ejaculate were not disturbed. Therefore, light regimen had a direct effect on the testicular production, but did not on the activity of accessory glands. Both excessive and lack of light exposure did not alter the motility, vitality and acrosomicresistance of spermatozoa, but resulted in abnormalities in sperm morphology due to an increased frequency of immature spermatozoa and of spermatozoa with secondary anomalies on the tail. These results indicated that, at testicular level, despite the low sperm production, the light regimen did not impair the germ cell differentiation, whereas at epididymal level it induced some disturbances in tail maturation.

P-058. Vitrification of refrozen human blastocysts

Haigo K., Fukunaga N., Chiba S., Kato S. and Kyono K.

Department of Gynecology and Urology, Lady's Clinic Kyono, 3–8–6 Omiya, Furukawa City, Miyagi 989-6221, Japan

Introduction: The aim of this study was to examine the survival and development ability of refrozen-thawed embryos and to compare the two methods of cryopreservation: straw and cryoloop.

Materials and methods: In this study, all embryos were derived from extra frozen embryos of patients who had given informed consent. Experiment 1: The thawed embryos, frozen at 2PN and ~2–5cell, were respectively cultured for 48 h and 24 h in
P-1 medium. Embryos were then transferred into blastocyst medium. Embryos were observed at 48 h after transfer into blastocyst medium. The protocols for freezing and thawing were performed as described by Testart *et al.*. Experiment 2: Morulae and blastocysts developed from frozen-thawed embryos were recryopreserved by vitrification using straw or cryoloop. The methods for refrozen-thawed using straw were performed as established by Yokota *et al.* and using cryoloop were modified as described by Lane *et al.*; we used modified-HTF containing 20% serum substitute supplement (SSS) as base medium for vitrification. Refrozen–thawed embryos were assessed for survival rate and hatching blastocyst rate by morphology at 24 h and 48 h.

Results: Experiment 1: The rate of survival and development beyond grade 3 blastocyst (Gardner's classification for blastocyst) of frozen–thawed embryos were not significantly different between the embryos frozen at 2PN (68.2 and 6.8% respectively) and ~2–5 cell (56.8 and 9.1% respectively). Experiment 2: Although the survival rate and hatching blastocyst rate of refrozen–thawed blastocysts after culture of 24 h and 48 h was not significantly different between cryoloop and straw, the rate was higher in cryoloop (50.0 versus 25.0%) than straw (84.6 versus 38.5%).

Conclusions: This study demonstrated the high ability of refrozen-thawed blastocysts to survive. The average survival rate for blastocysts was ~70%. Cryoloop was more effective for refrozen–thawed methods than straw. It is necessary to investigate further the chromosomal abnormality of refrozen-thawed embryos using fluorescent in-situ hybridization (FISH) and/or polymerase chain reaction (PCR).

P-059. Increased pregnancy rates following preimplantation genetic diagnosis in selected patients with poor prognosis

Bahçe M.[1,2], Budak E.[1], Özcan C.[1], Karagözoğlu S.[1], Yorulmaz Ö.T.[1], Akyuva R.[1], Öcal A.[1], Çağdaş A.[1], Imirzalioglu N.[2] and Karaman Y.[1]

[1]IVF and Reproductive Genetics Center, Istanbul Surgery Hospital, Istanbul and [2]Department of Medical Genetics, Gülhane Military Medical Academy and School of Medicine, Ankara, Turkey

Introduction: To investigate the effect of preimplantation genetic diagnosis (PGD) for aneuploidy screening on implantation, clinical pregnancy and miscarriage rates in a selected group of IVF patients with an expected poor outcome.

Materials and methods: The study group consisted of 16 infertile patients who consented for PGD with the following indications. Repeated implantation failure and male factor ($n = 3$), repeated implantation failure and advanced maternal age ($n = 3$), severe male factor ($n = 3$), repeated implantation failure and bad embryo quality ($n = 2$), repeated pregnancy losses and previous chromosomal pathology ($n = 2$), advanced maternal age ($n = 2$), repeated implantation failure and pregnancy losses ($n = 1$). Ovarian stimulation was accomplished by exogenous gonadotrophin administration following a desensitization protocol with daily injections of a gonadotrophin-releasing hormone analogue. At 36 h after injection of human chorionic gonadotrophin, oocytes were retrieved transvaginally under ultrasound guidance. Metaphase II oocytes were fertilized by intracytoplasmic sperm injection. On day 3, blastomere biopsy and fluorescent in-situ hybridization (FISH) were carried out on 124 embryos from 16 infertile patients. All of the biopsied embryos were grade I-II embryos with six blastomeres or more. FISH was performed to detect aneuploidy for chromosomes 13, 16, 18, 21 and 22. Only embryos classified as normal after PGD were transferred on day 4.

Results: Average maternal age was 33.43 (range 28–42 years) years. Following FISH, 62 (50%) embryos were detected as normal, 54 (43.5%) embryos as abnormal and in eight (6.5 %) embryos no result was obtained. The abnormal embryos were classified as follows: aneuploidies ($n = 43$, 79.6%), polyploidies and haploidies ($n = 11$, 20.4%). All of the 16 patients had at least one embryo transferred. A total of 50 embryos were transferred, with an average transferred embryo number of 3.1 (range 1–5). Clinical pregnancy was achieved in eight (50%) patients with an implantation rate of 22% (11/50). Biochemical pregnancy occurred in one patient, while no pregnancy occurred in seven patients. So far, no pregnancy loss or absent fetal heart movement has occurred in seven pregnancies exceeding 11 weeks pregnancy, while one patient is currently at 6 weeks of gestation.

Conclusion: Chromosomal abnormalities are believed to be a major factor contributing to low implantation and pregnancy rates in patients expected to have poor IVF outcome or who are at increased risk of early pregnancy loss. Preliminary results of PGD at our centre suggest an improved IVF outcome, with higher clinical pregnancy and lower miscarriage rates in a subgroup of patients with poor prognosis. Transfer of normal embryos as detected by PGD may improve implantation and pregnancy rates in infertile couples at risk of poor IVF outcome, recurrent abortion or who are at increased risk of conceiving a chromosomally abnormal fetus.

P-060. Risk of a low number of retrieved oocytes at first IVF treatment following unilateral ovariectomy and IVF

de Boer E.J.[1,2], den Tonkelaar I.[2], Burger C.W.[3], te Velde E.R.[4] and van Leeuwen F.E.[1]

[1]Netherlands Cancer Institute, Amsterdam, [2]International Health Foundation, Utrecht, [3]Department of Obstetrics and Gynaecology, Division of Gynaecology and Oncological Gynaecology, University Hospital Rotterdam and [4]Department of Reproductive Endocrinology and Fertility, Division of Obstetrics, Neonatology and Gynaecology, University Medical Center Utrecht, The Netherlands

Introduction: The 10–15 year period preceding the last menstrual cycle is characterized by an accelerated oocyte loss. It is postulated that women who were unilaterally ovariectomized at an early age reach this critical threshold earlier than non-ovariectomized women. Early ovarian depletion is also likely to be reflected by a low number of retrieved oocytes after gonadotrophin stimulation for IVF treatment.

Materials and methods: We used data from a nationwide Dutch cohort study (OMEGA) of ovarian stimulation for IVF and subsequent gynaecological diseases ($n = 26\,428$) to study the risk of retrieving a low number of oocytes at first IVF treatment following unilateral ovariectomy. We also examined whether women who were unilaterally ovariectomized >3 years before IVF treatment ('early') had more often a low number of retrieved oocytes at their first IVF cycle ($\leqslant 3$ oocytes) than women who were unilaterally ovariectomized <3 years before IVF treatment ('late'). A total of 143 early ovariectomized, 107 late and 7503 non-ovariectomized women were included in the analysis. Detailed information about ovariectomy and IVF treatment was obtained from the medical files. Logistic regression was used to calculate odds ratios (OR) and 95% confidence intervals (CI).

Results: The median numbers of retrieved oocytes were 5, 6 and 8 among early ovariectomized, late ovariectomized and non-ovariectomized women, respectively [$P < 0.0001$ (early versus non-ovariectomy), $P < 0.0001$ (late versus non-ovariectomy)]. After adjustment for age at first IVF treatment, year of first IVF treatment, dosage of human menopausal gonadotrophin/FSH, treatment hospital and subfertility diagnosis, the OR for a low number of retrieved oocytes at first IVF treatment was 2.1 (95% CI 1.6–2.8) for unilaterally ovariectomized women as compared to non-ovariectomized women. For early and late ovariectomized women, the adjusted OR were 2.0 (95% CI 1.3–3.1) and 2.1 (95% CI 1.5–3.1) ($P = 0.74$).

Conclusions: These data suggest that early ovariectomized women are not at a higher risk for a low number of retrieved oocytes at first IVF treatment as compared to late ovariectomized women.

P-061. Reduction of volatile organic compounds in incubators by activated charcoal

Weissenborn U.[1], Möller M.[2] and Neulen J.[1]

[1]Universitäts-Frauenklinik für Gynäkologische Endokrinologie und Reproduktionsmedizin and [2]Institut für Hygiene und Umweltmedizin, Medizinische Fakultät der RWTH Aachen, Germany

Introduction: The impact of air quality in assisted reproduction laboratories on IVF results has been established for some years. Thus, efforts have been spent on removing volatile organic compounds (VOC) from laboratories and incubators to prevent potential harmful effects on gametes and embryos. Commercially available air filter systems for laboratories and incubators are used to improve the quality of the atmosphere.

Materials and methods: Measurements of air contaminants were performed in neighbouring IVF/intracytoplasmic sperm injection laboratories and in two identical incubators in each of the laboratories. Incubators were gassed with 5% CO_2 in air with humidified atmosphere and cleaned regularly. A commercially available air filter system (Coda In-line Filter and Coda Incubator Filtration Unit; genX international) was installed in the CO_2 hose and in one of the incubators. Measurements of VOC were performed 6 weeks after installation. The efficacy of activated charcoal in reducing air contaminants was analysed by placing Petri dishes (diameter: 94 mm, TC quality), filled with ~20 g activated charcoal, at different positions in the incubators. Measurements were done after 2 weeks. Air quality in the laboratory was analysed in parallel. VOC were sorbed on Tenax GR cartridges either by diffusive sampling or by an active sampling method, using suction pumps equilibrated to an air flow rate of 10 ml/min. The samples were stored until analysis in the original cartridges with teflon-lined screw caps. For the analysis the cartridges were heated in a thermal desorption unit under helium purge and the desorbed VOC were collected on a cold trap filled with Tenax-TA. The collected organics were flash-injected onto a high-resolution GC/MS system and monitored in full-scan mode.

Results: Analysis of VOC showed that composition and quantity of air contaminants were different in the laboratory air as compared to the incubators. Whereas in the laboratory atmosphere only low concentrations ($\mu g/m^3$) of aromatic and aliphatic carbohydrates were detected, in the incubators these hydrocarbons and additional compounds were present at concentrations up to mg/m^3. These compounds are organic solvents which are present, for example in plastic materials of disposables for cell culture. After installing the commercially available filter system, concentrations of VOC in the incubator were higher than without filters, which is possibly due to plastic materials of filter cartridges as an additional source of VOC. Use of activated charcoal clearly reduced VOC by about 75% in the incubator. The reduction of VOC by activated charcoal was confirmed by measurements in the second incubator. CO_2 as a source of VOC could be excluded.

Conclusions: The results show that application of activated charcoal is an efficient uncomplicated means of reducing VOC at low costs.

P-062. Is ICSI helpful in couples with idiopathic infertility?

Wyns C., Demylle D., Vandermonde J., Dolmans M.M., Nisolle M. and Donnez J.

Department of Gynecology, Catholic University of Louvain, Brussels, Belgium

Introduction: The outcome of intracytoplasmic sperm injection (ICSI) varies depending on the indication for treatment. We therefore analysed data in a particular group of patients with idiopathic infertility. We evaluated the results of IVF-embryo transfer in couples with unexplained infertility and determined if there is an improvement of their infertility prognosis with the ICSI procedure.

Materials and methods: A total of 107 couples with unexplained infertility proved by standard work-up including a laparoscopy underwent 181 IVF cycles with normal insemination (control group) and were compared with our study group. The study group consisted of 30 IVF cycles, first attempt for idiopathic infertility. Their oocytes were randomly evaluated in the case of normal insemination or ICSI.

Results: See Table I.

Table I.

	Control group $n = 181$ cycles	Study group $n = 30$ cycles	
	Normal insemination	Normal insemination	ICSI
Age (years)	33.0 ± 4.4	31.6 ± 3.7	31.6 ± 3.7
Infertility duration (years)	4.8 ± 3	4.8 ± 1.3	4.8 ± 1.3
No. of metaphase II oocytes (mean ± SD)	9.6 ± 5.3	6.37 ± 3.5	6.8 ± 2.7
Fertilization rate (%)	57.3	62.3	64.2
Implantation rate (%)	21.1	20.6	
Pregnancy rate per transfer (%)	36.7	46.7	

Conclusions: No improvement was observed when ICSI was carried out in patients with idiopathic infertility. In idiopathic infertility, only 10.5-16.6% of cases did not demonstrate fertilization. The proposal of ICSI in cases of idiopathic infertility is questionable. In our series, the number of cycles when no fertilization (0%) of the oocytes occurred was 10.5 and 16.6% in the case of normal insemination. In the case of ICSI, no total fertilization failure was observed. Our data suggest that there is no improvement in the IVF outcome in terms of fertilization, implantation and pregnancy rate between our control group with normal insemination, our study subgroup with normal insemination and our study subgroup with ICSI.

P-063. Ultrasound measurement of the utero-cervical angle during embryo transfer | a prospective controlled study

Sallam H., Agameya A., Rahman A., Ezzeldin F. and Sallam A.

Department of Obstetrics and Gynaecology, the University of Alexandria, and the Alexandria Fertility Center, Alexandria, Egypt

Introduction: The aim of this study was to evaluate the technique of measuring the utero-cervical angle by transabdominal ultrasound during embryo transfer in order to guide the embryo transfer catheter inside the uterine cavity.

Materials and methods: A total of 198 couples undergoing embryo transfer after IVF or intracytoplasmic sperm injection (ICSI) were alternately allocated to one of two study groups, each group consisting of 99 couples. In the first group (control group), embryo transfer was performed without ultrasound guidance, while in the second group, the embryo transfer catheter (Frydman TDT) was moulded according to the utero-cervical angle measured by transabdominal ultrasound. There were no statistically significant differences between both groups regarding the mean age, the mean duration of infertility or the mean number of embryos transferred.

Results: The results show that the incidence of difficult transfers was 2.0% (2/99) in the ultrasound-guided group compared to 8.1% (8/99) in the control group ($\chi^2 = 3.791$, $P = 0.0515$). The pregnancy and

implantation rates were 26.3% (26/99) and 10.3% (43/419) in the ultrasound-guided group compared to 18.2% (18/99) and 9.8% (25/256) in the control group, respectively. These differences are not statistically significant but in order to reach statistical significance the least numbers needed to study (NNT) were calculated. In order to improve the pregnancy rate from 18% to 28%, accepting a 90% probability of finding a true difference and taking $P < 0.05$, the NNT is 332 patients in each arm of the study. In order to improve the implantation rate from 10 to 20%, the least NNT is 217 patients in each arm of the study.

Conclusions: Measuring the utero-cervical angle by transabdominal ultrasound during embryo transfer was associated with increases in pregnancy and implantation rates, which did not reach statistical significance. The study is ongoing.

P-064. Fresh versus frozen embryo transfers in oocyte donation cycles: what is the difference?

Haigh C., Shah O., Salha A., Naeem T. and Sharma V.

Assisted Conception Unit, St James's University Hospital, Leeds LS9 7TF, UK

Introduction: Oocyte donation is an effective treatment for women lacking functional ovaries and those at risk of transmitting a genetically inherited condition. However, oocyte donation cycles using fresh embryos entails exquisite timing, and occasionally, balancing the donors' cycle with the recipients' can be challenging. The availability of cryopreserved embryos prior to the treatment cycle allows greater flexibility in the planning of oocyte donation cycles, allows embryo quarantine to minimize risk of transmission of infectious diseases and eliminates the unpredictable ovarian response of the donor. The objective of this study was to compare the outcomes of oocyte donation cycles using fresh embryos compared with those having frozen–thawed embryos replaced.

Materials and methods: In this retrospective analysis, recipients of fresh oocyte donation cycles were age-matched with those receiving frozen embryo transfer treatment (FET). All patients in the study received the same long protocol of ovarian down-regulation with gonadotrophin-releasing hormone analogues (GnRHa) (either buserelin acetate or Nafarelin intranasal spray) commencing on the first day of the menstrual cycle. After 21 days, pituitary desensitization was assessed using transvaginal ultrasonography to ensure that the endometrial thickness was $\leqslant$5 mm. Thereafter, pituitary desensitization was maintained and endometrial priming was achieved using oral oestradiol valerate. Once an endometrial thickness of $\geqslant$9 mm was achieved, the GnRHa was stopped, intramuscular progesterone was commenced and embryo transfer planned 4 days later.

Results: A total of 68 patients (mean age 38.6 $\pm$ 4.8) who had 73 oocyte donation treatment cycles were included in the analysis. The overall clinical pregnancy rate per cycle was 35.6%. Twenty-nine patients (mean age 39.2 $\pm$ 4.4) underwent 33 oocyte donation cycles using frozen–thawed embryos while the remaining 39 patients (mean age 38.1 $\pm$ 5.1) had 40 fresh oocyte donation treatments. The mean number of embryos replaced in both groups was comparable (2.3 $\pm$ 0.7 in the frozen–thawed embryos group compared to 2.5 $\pm$ 0. 5 in the fresh group). The outcome of the two treatment cycles is illustrated in Table I. It is evident that there was no statistically significant difference between the clinical pregnancy rates per cycle or per patient in either group.

Table I. Outcome of oocyte donation cycles using fresh compared to frozen-thawed embryos

	No. of patients	No. of cycles	Clinical pregnancies	Clinical pregnancy rate/cycle (%)	Clinical pregnancy rate/patient (%)
Fresh embryos	39	40	15	37.5	38.1
Frozen embryos	29	33	11	33.3	37.9
Total	68	73	26	35.6	38.2

Conclusions: The use of cryopreserved embryos in oocyte donation cycles is not only more cost-effective than fresh cycles but also leads to a similar outcome. The usage of frozen-thawed oocyte donation cycles also facilitates better synchronization of the donor-recipient pair and eliminates the risk of unexpected cycle cancellation.

P-065. Blastocyst quality, pregnancy and multiple gestation rates. Is it feasible yet to transfer a single blastocyst to a selected patient population?

Rodriguez J.A., Crider-Pirkle S.S., Madden J.D., Guerami A.R., Douglas J.W., Barnett B.D., Rodriguez A.J., Bookout D.M., Chantilis S.J. and Meintjes M.

Presbyterian ARTS Program, 8160 Walnut Hill Ln., 6th Floor Perot Building, Dallas, TX 75231, USA

Introduction: High implantation rates are reported when transferring blastocysts. Blastocyst culture and transfer is frequently proposed as a possible remedy to the multiple pregnancy epidemic associated with assisted reproduction. However, even when transferring blastocysts, multiple gestation rates in excess of 50% may still result. The goal of this study was to evaluate the effect of blastocyst quality on the implantation, pregnancy and multiple gestation rates to identify a patient population-blastocyst quality combination with an optimum pregnancy/multiple gestation ratio.

Materials and methods: Blastocyst quality data were prospectively collected for 639 consecutive patients during a 2 year period. Patients were then divided into four groups (see Table I) based upon the number of good quality blastocysts transferred and analysed for implantation, clinical pregnancy and multiple pregnancy rates. Patient age (age groups; $\leqslant$34 years, 35–37 years, 38–40 years) was also taken into account. Only patients $\leqslant$40 years of age using homologous oocytes were included in this evaluation.

Results: When two good quality blastocysts were transferred in patients $\leqslant$34 years of age ($n = 163$) the clinical pregnancy and multiple gestation rates were 85.3 and 72% respectively. When only one good quality blastocyst was transferred in this age group ($n = 102$), the clinical pregnancy and multiple pregnancy rates were 71.6 and 35.6%.

Table I.

	Group 1	Group 2	Group 3	Group 4
No. of patients	172	179	271	17
No. of good quality embryos	0	1	2	3
Average age (range)	33.5 (26–40)	34.8 (22–40)	34.2 (23–40)	32.9 (27–39)
No. blastocysts transferred (average)	395 (2.30)	421 (2.35)	587 (2.17)	52 (3.06)
Clinical pregnancies (%)	68[a] (39.5)	108[b] (60.3)	206[c] (76.0)	10[a,b,c] (58.8)
Implantations (%)	99[a] (25.1)	177[b] (42.0)	335[c] (57.1)	14[a] (26.9)
Multiple pregnancies (%)	26[a] (38.2)	43[a] (39.8)	138[b] (67.0)	5[a,b] (50.0)

[a,b,c]Rows with no common superscript are different. χ^2; $P < 0.03$.

Conclusions: When two good quality blastocysts are transferred, exceptionally high implantation and clinical pregnancy rates can be obtained. However, the multiple pregnancy rate is also very high. When only one good quality blastocyst is transferred instead of two, the multiple pregnancy rate can be halved without a significant decrease in the clinical pregnancy rate in patients $\leqslant$34 years of age. Excellent pregnancy rates should be achievable from a single quality blastocyst transfer to these patients, resulting in the practical elimination of multiple pregnancies. See Table I.

P-066. Risk factors for miscarriage in an oocyte donation programme

Caligara C.[1], Camargo F.[1], Pinilla L.F.[1], Escudero E.[1], Navarro J.[1], Bolúmar F.[3], Simón C.[1,2], Pellicer A.[1,2] and Remohí J.[1,2]

[1]Instituto Valenciano de Infertilidad, [2]Department of Paediatrics, Obstetrics and Gynaecology, Valencia University School of Medicine, Valencia and [3]Public Health Department, Miguel Hernández University, Alicante, Spain

Introduction: The objective of this study was to identify clinical characteristics associated with a higher risk for miscarriage in an oocyte donation programme.

Materials and methods: Pregnancies achieved after oocyte donation between January 1, 1996 and June 30, 2000 were retrospectively studied. Analysed variables were patient's age, indication for oocyte donation, semen characteristics, received oocytes, fertilization technique, fertilization rate, number and day of transferred embryos, implantation rate, cycle number of oocyte donation, previous miscarriages in oocyte donation treatments, previous live births in oocyte donation cycles, previous spontaneous miscarriage, as well as donor's age and total number of retrieved oocytes. Logistic and multiple logistic regressions were used for statistical analysis.

Results: A total of 917 pregnancies were achieved in 816 patients, with a miscarriage rate of 25.2% (231/917 pregnancies). Logistic regression showed a higher risk for miscarriage with increasing maternal age (>40 years), progressive oocyte donation attempts (more than three), and miscarriage in a previous cycle or history of miscarriage in spontaneous conception. No correlation with miscarriage was found with female indication for oocyte donation, semen characteristics, number and day of transferred embryos, fertilization technique and oocyte donor's age. When analysed in multiple regression, statistical significance was preserved for maternal age at transfer above 40 years [odds ratio (OR) 3.96; 95% confidence interval (CI) 1.64–9.58] and for a sixth or higher cycle of oocyte donation (OR 2.39; 95% CI 1.05–5.46).

Conclusions: Maternal age above 40 years at transfer, and a sixth or higher cycle of oocyte donation were factors associated with a significantly higher risk of miscarriage in an oocyte donation programme.

P-067. Empty follicle syndrome: iatrogenic factors causing infertility

Sarac G.[1,2], Demircan A.[1,2], Karacan M.[2], Benhabib M.[2], Atasü T.[3] and Djahanbakhch O.[4]

[1]Kibele Üreme Sagligi Merkezi, Istanbul, Turkey, [2]International Hospital-Istanbul, [3]Cerrahpasa Tip Fakültesi and [4]London Medical Hospital College, UK

Introduction: Follicular aspiration without retrieval of any oocytes is a rare cause of infertility and a cause of embarrassment for an IVF team. Empty follicle syndrome (EFS) was first reported in 1986 and is still encountered sporadically in IVF programmes. The causes were attributed to incorrect injection of human chorinic gonadotrophin (HCG), or low activity of HCG batches. In this study we present our 11 years of experience with oocyte collection cycles and discuss possible iatrogenic factors.

Materials and methods: All patient data for the 11 years of IVF practice have been retrospectively analysed. The ovaries were stimulated mainly with long protocol and flare-up protocol. There were 2092 cycles of oocyte retrieval cycles in which HCG was used to trigger the final oocyte maturation. Oocyte collection was scheduled 34–36 h after the HCG injection. Patients who had ⩾4 follicles were included in this study. When there were no oocytes retrieved after the first aspiration, the follicles were flushed with medium at least 10 more times. After aspiration of 2–4 follicles without oocyte retrieval the needle was changed with a new one and a serum sample was obtained for HCG assessment. If serum HCG concentrations were undetectable, HCG was injected and the oocyte retrieval was postponed for 24 h.

Results: In 2092 oocyte retrieval cycles, EFS was encountered in five cases between 1990 and 1992 among 353 cycles. In four cases serum HCG concentrations were negative. On further investigation it was found that these patients had had only water injections by mistake. Serum HCG was positive in one patient and this patient had her HCG injection 24 h later than the scheduled time, and therefore had her oocyte retrieval 12 h after the injection. Two of the patients achieved pregnancy and delivery in their second attempts. After 1992 we used a strict programme of HCG injection protocol, ensuring that the patients had their injections at the scheduled time and in a correct manner, and changing the needle was set as a standard when indicated. After 1992 all cycles performed in good responders yielded oocytes. In 56 cases collection of oocytes was possible after changing the needle. In one case, oocytes were collected after a second HCG injection.

Conclusions: EFS is a rare cause of infertility. Our knowledge of follicular development dictates that in the presence of a normal follicular development as confirmed with normal follicle growth, increasing serum oestradiol concentrations and endometrial thickness increase, there should be no empty follicles. In our experience empty follicles are the result of either incorrect HCG injection (injecting only water or incorrect timing) or a needle blocking factor. Needle block may not be a problem in patients with abundant follicles but it may be a disaster in patients with a small number of oocytes.

P-068. Day 2 versus day 3 embryo transfer in IVF and ICSI cycles

Marsella T., Di Girolamo R., Lagalla C., Salvatori M., Ciaccio I., Giulini S., Gallinelli A. and Volpe A.

Department of Gynaecological, Obstetrics and Pediatric Sciences, University of Modena, Modena, Italy

Introduction: Embryo prolonged culture has been proved to be a very important technique to allow couples, undergoing assisted reproduction treatment, to improve the chance of pregnancy and minimize their risks for multiple gestation. Nevertheless the management of prolonged embryo culture presents a number of difficulties connected to the embryo's ability to grow *in vitro* in synthetic media. Embryos are usually transferred into uterus at the 2–4-cell stage to minimize the in-vitro embryo degeneration or cytoplasmic fragmentation. The aim of this study was to compare the pregnancy rates obtained when selected embryo transfer was performed 48 or 72 h after intracytoplasmic sperm injection (ICSI) or IVF procedures.

Materials and methods: Ninety-five ICSI and 34 IVF procedures were performed between January 2000 and December 2000. All patients were stimulated with the combination of gonadotrophin-releasing hormone agonist (Enantone, Takeda; or Decapeptyl 3.75, Ipsen) and recombinant FSH (Gonal-F; Serono). Ovulation was induced by administration of a single dose of 10 000 IU human chorionic gonadotrophin (Profasi; Serono) 36 h before oocyte retrieval. After retrieval, all the recovered metaphase II oocytes were inseminated in human tubal fluid medium (Irvine Scientific), the fertilization checking was performed 18–20 h after IVF/ICSI and the zygotes were cultured in P1 medium (Irvine Scientific) until the transfer. Patients were randomly allocated to day 2 transfer (39 ICSI and 14 IVF) and day 3 transfer (56 ICSI and 20 IVF). Embryo quality and cleavage were daily evaluated.

Results: The results are summarized Table I.

Table I.

	ICSI (95 patients)		IVF (34 patients)	
	48 h (39 patients)	72 h (56 patients)	48 h (14 patients)	72 h (20 patients)
Female age (years)	35.3 ± 5.3	34.5 ± 3.9	35.4 ± 3.9	34.1 ± 2.9
Transferred embryos/patient	2.2 ± 1.1	2.7 ± 1.0	3.0 ± 0.6	2.7 ± 1.1
Good quality embryos (grade A/B)	64/86 (74.1)[a]	77/52 (50.6)[b]	40/43 (93)[c]	32/54 (59.2)[d]
Pregnancy rate/transfer	7/39 (18)[e]	8/56 (14)[f]	3/14 (21)[g]	9/20 (45)[h]

Values in parentheses are percentages.
a versus b, c vs d: $P < 0.001$; a versus c: $P < 0.05$; b versus d: not significant; f versus h: $P < 0.005$; e versus f, g versus h, e versus h: not significant.

Conclusions: (i) Some good quality day 2 embryos, when observed 24 h later, show developmental arrest. (ii) A higher pregnancy rate was obtained when embryo transfer was performed at day 3, at least in patients undergoing IVF. These findings seem to suggest that embryo morphology, when observed after only 24 h of culture, cannot be considered as an optimal parameter to select embryos for transfer.

P-069. Case report. Ehlers-Danlos syndrome: recurrent premature follicular rupture during gonadotrophin treatment for IVF not prevented by a gonadotrophin-releasing hormone antagonist (Cetrotide)

Kazem R.

Nottingham University Research and Treatment Unit in Reproduction (NURTURE), Division of Reproductive Medicine, School of Human Development, University of Nottingham, Floor B, East Block, Queen's Medical Centre, Nottingham NG7 2UH, UK

Introduction: Ehlers-Danlos syndrome (EDS) is a rare genetic connective tissue disorder, which features skin hyperextensibility, articular hypermobility and tissue fragility. Several pregnancy-associated complications have been described, including preterm rupture of the fetal membranes and premature labour due to cervical incompetence. Some gynaecological problems including genital prolapse, incontinence and dyspareunia have also been described. There is no published report on problems during ovarian stimulation in women with EDS. We describe a case where three successive cycles of ovarian stimulation resulted in premature loss of follicles.

Case Report: A 32 year old woman and her partner were referred to our Unit for surgical sperm retrieval and ICSI (intracytoplasmic sperm injection) treatment for male factor infertility due to congenital bilateral absence of the vas deferens. The female partner had a mild form of EDS and had previously undergone genetic counselling. The standard long protocol of ovarian stimulation was followed in the first and second cycle of ICSI treatment. A gonadotrophin-releasing hormone antagonist (GnRH; Cetrotide) was used in the third cycle of treatment. Each cycle was characterized by premature loss of ovarian follicles prior to the oocyte recovery procedure. During the first cycle of ICSI treatment, six large follicles of $\geqslant$17 mm diameter developed when human chorionic gonadotrophin (HCG) was administered. At oocyte recovery, 36 h after HCG, only four large follicles were present and only two oocytes were recovered despite multiple flushing of each follicle. Spermatozoa obtained by surgical retrieval were used for ICSI. Fertilization failed to occur. During the second cycle of treatment, seven large follicles of $\geqslant$17 mm diameter and two small follicles developed when HCG was administered. At oocyte recovery, 35 h after HCG, only two small follicles were visualised and no oocytes were obtained. In the third cycle, ovarian stimulation was commenced on day 3 of the menstrual cycle. Cetrotide (3 mg s.c. injection) was administered on day 7 of stimulation. Nine follicles developed (11, 12, 13, 14, 16, 17, 17, 18 and 21 mm diameters) on day 8 of stimulation when HCG was administered. Oocyte recovery was planned 34 h after HCG (72.5 h after Cetrotide). Only two large follicles (diameters 17 and 20 mm) were visualized and two oocytes were obtained. One oocyte was mature, this fertilized after ICSI with frozen-thawed sperm obtained previously, and one grade 1 4-cell embryo was transferred into the uterus. Pregnancy did not occur.

Discussion: This case report is, to our knowledge, the first report of premature loss of follicles occurring in three successive cycles of ovarian stimulation in a woman with EDS. We can presume that the premature ovulation was probably due to some defect in the collagen/connective tissue in the wall of the follicles. Premature ovulation during ovarian stimulation is a rare occurrence and has not been reported when a GnRH antagonist has been used. Ovulation may occur prematurely, as early as 34 h following withdrawal of GnRH agonists, but the antagonist has a profound suppressive effect that lasts for a minimum of 96 h. Our patient had the oocyte collection well within the 96 h mark. This cycle in particular and the recurrence of premature rupture of follicles in three cycles lends support to the influence of mechanical factors rather than hormonal events in the pathogenesis. It is thought that collagen changes in patients with EDS are responsible for events such as premature rupture of fetal membranes in pregnancy, premature labour and haemorrhagic events, but premature rupture of follicles has not been described. There are no data on scan findings monitoring ovarian follicles during natural cycles of patients with EDS. However, the literature on gynaecological problems in EDS is scanty and such problems may be under-reported.

P-070. Pelvic inflammatory disease in oocyte retrieval

Moeini Ashraf, Amid vida and Sadeghi Maria

Royan Institute, P.O.Box 19395-4644, Tehran, Iran

Introduction: Medical and surgical complications are an inevitable aspect of assisted reproductive techniques; pelvic inflammatory disease (PID) is considered to be a rare complication. In a retrospective study of 5958 IVF or ICSI cycles performed in our institute, we extracted 10 cases of PID for which predisposing and aetiological factors have been identified.

Material and methods: A total of 5958 oocyte retrievals among patients referred to Royan Institute were included. They were prepared for IVF or ICSI using either ejaculated or surgical sperm retrieval during 1994–2000. All patients underwent ultrasonically guided vaginal oocyte retrieval by a semi-sedation technique and pretreated with clomiphene citrate, GnRHa and human menopausal gonadotrophin protocol. Vaginal preparation was performed by sterile distilled water. The patients were discharged on the day of aspiration.

Results: In all, 2887 oocyte retrievals for IVF-embryo transfer and 3300 oocyte retrievals for ICSI were performed. PID occurred in 10 patients. Eight of 10 had endometriosis. No pregnancy, biochemical or clinical, was recorded. Six of 10 were being treated as outpatients for PID. Therapeutic operative procedures were required in four cases and two patients underwent therapeutic laparoscopy, four had endometriosis.

Conclusions: Transvaginal oocyte retrival may introduce bacteria into the intraperitoneal cavity. If we use standard procedure its complications will be rare. The past history of endometriosis may increase the risk of PID. In the presence of PID, endometriosis is perhaps more likely to be present than bowel perforation. To prevent total failure, cryopreservation and embryo transfer in subsequent cycle should be considered in cases with PID. The use of meticulous vaginal preparation with povidone iodine, minimal number of penetrations during the procedure, use of prophylactic antibiotics in patients with a history of endometriosis may be useful at the time of oocyte retrival.

P-071. Infertile patients asking for treatment in their late reproductive life-span: which treatment is best?

Tabanelli C., Ferraretti A.P., Iammarrone E., Magli M.C., Barnabè D. and Gianaroli L.

S.I.S.M.E.R, Reproductive Medicine Unit, Bologna, Italy

Introduction: Infertile patients asking for treatment in their late reproductive life-span are becoming more frequent in IVF centres. If they already show hormonal and clinical signs of a premenopausal status, the only treatment to propose is oocyte donation. If they are still normovulatory women, a doubt often arise whether it is better to try an IVF cycle or to allow them to enter the oocyte donation programme because of the poor prognosis of IVF related to age. In this study we present the clinical data obtained in the last 4 years of activity in our centre where conventional IVF and intracytoplasmic sperm injection (ICSI) procedures, oocyte donation programme using young infertile donors undergoing IVF and preimplantation genetic diagnosis (PGD) of aneuploidy are available. The age upper limit for treatment is 51 years.

Materials and methods: To all patients >40 years entering our centre, the following treatment was proposed. Premenopausal women were treated only by oocyte donation. To normovulatory women was first suggested IVF (or ICSI) with PGD, then oocyte donation in cases of failure (no response, no embryo development, no euploid embryos available for transfer).

Results: A total of 115 premenopausal women (age 45.7 $\pm$ 3 years) underwent 191 embryo transfers with donated oocytes, showing a pregnancy rate (PR) of 21.4 per embryo transfer and 37% per patient. 482 IVF and ICSI cycles were performed in normovulatory patients, showing an acceptable PR (20%) in the 40–43 year old group and very poor results in $\geqslant$44 year old group. In addition, as previously published, the

40–43 year old patients who underwent PGD, showed a high PR per embryo transfer. Previous failed IVF patients had acceptable PR entering oocyte donation (18.4% per embryo transfer, 37% per patient). Data are presented in Table I. The implantation rate was not different among the group, a part of ⩾44 year old group undergoing IVF. A lower abortion rate was present in women undergoing oocyte donation because of infertility only due to the perimenopausal state compared to patients undergoing treatment for additional infertility problems (IVF failure), even where oocyte donation was not able to decrease the risk of early abortion. The uterine factor can have a more important role in these patients even if they are younger.

Table I.

Table	IVF-ICSI		Oocyte donation	
	40–43 years	>44 years	Previous IVF	Perimenopausal state
No. of patients	334	123	101	115
No. of cycles	361	121	211	208
No. of cancelled cycles	89	59	-	-
No. of transfers	199	28	201	191
No. of embryos/embryo transfer	1.1 ± 0.8	0.5 ± 0.9	1.5 ± 0.9	1.6 ± 1
No. of pregnancies (% per embryo transfer)	38 (20)	1 (3.6)	37 (18.4)	41 (21.4)
Implantation rate (%)	12.9	2	13.6	14.1
No. of miscarriages (%)	14 (37)	-	13 (35)	10 (24.7)

Discussion: When infertile old patients are asking for treatment, oocyte donation should be proposed as the first approach when age is >43 years even for normovulatory cycles. To propose oocyte donation as the first treatment in 40–43 year old infertile women still normovulatory does not offer them significantly higher live birth rate per embryo transfer, and an IVF attempt with PGD should first be performed to give them the last chance to have their own offspring.

P-072. Concentration of serum FSH during ovarian stimulation is dependent on body weight and body mass index

Miller K.F. and Behnke E.J.

Center for Reproductive Studies, Christ Hospital, Cincinnati and Section of Reproductive Endocrinology and Infertility, Cleveland Clinic Foundation, Cleveland, Ohio, USA

Introduction: Ovarian stimulation with multi-follicular growth is routine for assisted reproduction treatment. The ovarian response to a fixed dose of gonadotrophin is extremely variable among women. One variable that, to date, has received little attention is the role of body weight. Typically a dose expressed as a number of ampoules of medication is prescribed to effect ovarian stimulation without regard to patient body weight. This study examined serum concentrations of FSH and clinical outcome after 4 days of treatment with a fixed dose of gonadotrophin.

Materials and methods: Ovarian stimulation was begun after pituitary suppression with luprolide acetate was confirmed by oestradiol of <50 pg/ml, absence of ovarian follicles and a thin endometrium. Patients received 300 IU gonadotrophin in the evening for each of 4 days. A serum sample was drawn on the morning of the 5th day for oestradiol determination and subsequent individualization of the stimulation. Samples were stored frozen until serum FSH was determined by chemiluminescent assay (DPC Immulite; Diagnostic Products, Inc., Los Angeles, CA, USA). All samples were run within the same assay. Data were analysed on a body weight and body mass index (BMI) basis by analysis of variance and χ^2-test as appropriate.

Results: Serum FSH on day 5 was inversely related to both body weight and BMI. Analysis on a BMI basis showed smaller variation than weight alone and is presented here. Patients with either very low or very high BMI had lower pregnancy and implantation rates.

Table I.

	Body mass index				
	<20	20–25	25–30	>30	*P*
Starts (*n*)	39	128	55	27	
Age (years)	32.5 ± 3.4	33.1 ± 3.9	33.0 ± 4.1	34.9 ± 3.9	NS
Height (cm)	165 ± 7	165 ± 7	164 ± 7	165 ± 6	NS
Weight (kg)	51 ± 4	60 ± 6	72 ± 6	95 ± 16	< 0.0001
FSH (mIU)	13.6 ± 4.3	12.2 ± 3.4	10.5 ± 2.7	8.3 ± 2.6	< 0.0001
Oestradiol, day 5 (pg)	254 ± 161	243 ± 179	265 ± 185	183 ± 116	NS
Retrievals (*n*)	36	116	49	24	
Ampoules	36 ± 13	37 ± 12	34 ± 9	39 ± 11	NS
Length (days)	11.1 ± 1.6	11.0 ± 1.4	10.8 ± 1.4	11.2 ± 1.5	NS
Peak oestradiol (pg)	2432 ± 904	2338 ± 1086	2410 ± 1049	1787 ± 847	0.07
Oestradiol per oocyte	221 ± 104	207 ± 106	190 ± 84	230 ± 94	NS
Oocytes	12.6 ± 5.5	12.8 ± 6.6	14.2 ± 6.9	8.7 ± 4.7	0.08
Fertilized (%)	62 ± 22	58 ± 23	56 ± 22	63 ± 24	NS
Transfers (*n*)	36	99	43	23	
Embryos/ET	2.5 ± 0.7	2.7 ± 0.7	2.7 ± 0.6	2.8 ± 1.0	NS
Pregnant	10 (28)	45 (46)	21 (49)	6 (26)	< 0.01
Implanted	14 (16)	72 (27)	30 (26)	7 (11)	0.005

ET = embryo transfer; NS = not significant.

Conclusion: The concentration of FSH in serum after administration of a fixed dose varies with body weight and BMI. In that the body weight of this group of patients varied by nearly 3-fold, adjustment of the dose of gonadotrophin on a body weight basis may result in a more homogeneous response to stimulation.

P-073. Does granulosa cell function determine oocyte developmental potential in women undergoing ovarian stimulation for IVF/ICSI? The importance of follicular size

Tozer A.J.[1,2], Iammarrone E.[2], Nica R.A.[2], Gillott C.M.Y.[1], Al-Shawaf T.[1], Lower A.M.L.[1], Grudzinskas J.G.[1,2] and Iles R.[2]

[1]Fertility Centre, St Bartholomew's and The London Hospitals Trust, London EC1A and [2]The Williamson Laboratory, Queen Mary and Westfield College, St Bartholomew's Hospital, London EC1A, UK

Introduction: An interdependence exists between the oocyte and its surrounding granulosa cells (GC) although the respective roles of the oocyte and GC in controlling growth and maturation of the follicle have not been fully elucidated. This prospective study was designed to evaluate the luteinized GC population of individual follicles, with respect to number and steroidogenic activity in follicular fluid in culture *in vitro*, relative to the retrieval and subsequent fertilization of the oocyte in follicles of different sizes.

Materials and methods: Four to six follicles per patient were measured prior to aspiration at the time of oocyte retrieval, following ovarian stimulation for IVF/intracytoplasmic sperm injection. The follicular fluid was aspirated in a standardized manner by one investigator and the follicles flushed to maximize GC retrieval. Luteinized GC were isolated using a Percol gradient, counted and plated for culture (10 000 cells per well in 24-well plates). Culture medium was removed and stored for assay at 24, 72 and 120 h. Progesterone was measured in the follicular fluid and cultured media using a commercial enzyme-linked immunoassay and oestradiol was measured in the follicular fluid using a commercial radioimmunoassay.

Results: A total of 257 follicles were aspirated from 50 women, yielding 196 oocytes from which 157 embryos were derived. Statistically significantly more oocytes were retrieved from follicles of 16–24.9 mm in diameter compared to smaller and larger follicles (84 versus 65.4 versus 73.3% respectively; $P < 0.05$) although the fertilization rate was positively correlated with follicle size. A positive correlation was seen in the number of GC and the follicle size although no differences were seen in GC number relative to oocyte retrieval and fertilization. Follicular fluid concentrations of progesterone and oestradiol showed a positive correlation with follicle size ($P < 0.0001$) although not with GC number. Progesterone production *in vitro* was statistically significantly less in

follicles <15.9 mm in diameter at 72 and 120 h than in follicles 16–24.9 and >25 mm ($P < 0.05$)

Conclusions: GC appear to function differently as the follicle size increases, probably reflecting a more mature population of cells with a greater steroidogenic capacity. Rates of oocyte retrieval and fertilization appeared to be independent of GC in terms of oestradiol and progesterone production in follicles of >16 mm but statistically significant differences were seen in smaller follicles.

P-074. Defragmentation of moderate and severe forms of fragmented pre-embryos improve implantation rates in IVF-embryo transfer

Savić B.[1], Milačić D.[2], Petronijević M.[2] and Vrzić S.[2]

[1]Belgrade Fertility and Sterility Centre (BEFES), Belgrade and [2]Institute for Obstetrics & Gynaecology, Clinical Centre of Serbia, Belgrade, Yugoslavia

Introduction: It is well documented that almost 70% of pre-embryos have some degree of fragmentation, and that 10% of all pre-embryos have severe forms of fragmentation. As fragmented pre-embryos show lower implantation rate, some authors performed defragmentation with intention to improve the quality of pre-embryos and IVF results. The aim of this study was to compare implantation and pregnancy rates in patients with defragmented and fragmented-intact pre-embryos.

Materials and methods: During a 6 month period (January-July 2000), we performed defragmentation of 18 pre-embryos. Eleven of them were with severe fragmentation (≥20% of fragments) and seven cases with moderate fragmentation (10–20% of fragments). Control group was represented with 23 pre-embryos with severe and 17 pre-embryos with moderate fragmentation. Defragmentation was performed only in pre-embryos with free fragments located in perivitelline space.

Results: Implantation rate with defragmented pre-embryos (≥20% of fragments) was 13.5%, and with intact-fragmented pre-embryos from the same group 7%. Implantation rate in defragmented pre-embryos with 10–20% of fragmentations was 16.8%, and in intact-fragmented pre-embryos from the same group 9.7%. Difference in implantation rates between defragmented and intact pre-embryos from the same group was significant ($P < 0.05$). Comparing pregnancy rates between the same categories of defragmented and intact-fragmented pre-embryos, there was no significant difference, although pregnancy rates were higher in defragmented pre-embryos. Pregnancy rate in defragmented pre-embryos with severe fragmentation was 17%, and in 'intact' pre-embryos 13.6%. In defragmented pre-embryos with moderate fragmentation, pregnancy rate was 25.3% and in 'intact' pre-embryos was 19.2%.

Conclusions: Defragmentation in cases of severely and moderately fragmented pre-embryos improves implantation, but not pregnancy rate. According to our initial experience this procedure should be improved and reserved for patients who have only pre-embryos with severe fragmentation.

P-075. Relationship among multiple pregnancies, embryo quality and age of the patient

Herrer R., Aragonés M., Cabañes I., Isaza V., Mínguez Y. and Simón C.

IVI Madrid, C/Santiago de Compostela, 28035, Madrid, Spain

Introduction: We aimed to study the relationship between multiple pregnancies, quality of the transferred embryos and age of the patients.

Materials and methods: This was a retrospective study of 233 cycles of IVF/intracytoplasmic sperm injection in which three embryos/cycle were transferred on day 2. Cycles were distributed in three age groups: group A (≤33 years), group B (34-36 years) and group C (≥37 years).

Results: Pregnancy, implantation and multiple pregnancy rates are summarized in Table I. According to the number of good quality embryos transferred, same variables were evaluated: grade 1 embryos (G1): 4 cells, grade 1 of fragmentation, grade 1 of symmetry between blastomeres; grade 2 embryos (G2): 4, 5 or 6 cells, grade 1 or 2 of fragmentation and/or symmetry (Table II). Table III shows the data when good quality embryos (G1+G2) were grouped together.

Table I.

	Group A (≤33 years)	Group B (34–36 years)	Group C (≥37 years)
No. of cycles	115	74	44
Pregnancy rate (%)	65 (75/115)[a]	45 (34/74)[a,b]	43 (18/44)[a,b]
Implantation rate (%)	36 (125/345)[c]	23 (50/222)[c,d]	19 (25/132)[c,d]
Multiple pregnancy (%)	53 (40/75)[e]	41 (14/34)[e]	39 (7/18)[e]

[a]$P < 0.05$; [b]not significant (NS); [c]$P < 0.05$; [d]NS; [e]NS (χ^2-test).

Table II.

	Group A			Group B			Group C		
	Preg. (%)	Mult. (%)	Impl. (%)	Preg. %	Impl. (%)	Mult. (%)	Preg. (%)	Impl. (%)	Mult. (%)
0 G1	65 (35/54)	36[a]	51	49 (19/39)	24[a]	42	32 (9/28)	16[a,d]	33
1 G1	61 (17/28)	21[c]	59	50 (8/16)	25	37	37 (3/8)	12[e]	33
2 G1	60 (12/20)	37	67	53 (6/13)	13	33	100 (4/4)	50[e]	50
3 G1	84 (11/13)	46[b,c]	36	17 (1/6)	11[b]	25	50 (2/4)	33[d]	50

[a,b,c,d,e]$P < 0.05$.

Table III. G1+G2

	Group A			Group B			Group C		
	Preg. (%)	Mult. (%)	Impl. (%)	Preg. %	Impl. (%)	Mult. (%)	Preg. (%)	Impl. (%)	Mult. (%)
0G1+G2	80 (4/5)	40	25	25 (1/4)	17	100	0 (0/5)	-	0
1G1+G2	33 (2/6)	11	0	36 (4/11)	18	50	50 (4/8)	12	25
2G1+G2	66 (12/18)	26	25[b]	33 (3/9)	17	0	67 (4/6)	28	25
3G1+G2	65 (56/86)	40[a]	66[b]	52 (26/50)	26[a]	42	42 (10/24)	21[a]	50

[a,b]$P < 0.05$.

Conclusions: Young patients (≤33 years) show excellent pregnancy rate and implantation rate when two or three good quality embryos are transferred. Transferring three good quality embryos in this unique subgroup of patients does not improve pregnancy rate, but the multiple pregnancy rate rises considerably. A prospective randomized trial is on the way to confirm this retrospective study.

P-076. Hydroxyethyl starch (HSS) – a novel treatment for mild to moderate ovarian hyperstimulation syndrome: efficacy and cost-effectiveness analysis

Gamzu R., Winkler N., Botchan A. and Lessing J.B.

Gynecology Department, Lis Maternity Hospital, Tel-Aviv Sourasky Medical Center, 6 Weizman St, Tel Aviv, 64239 and Sackler School of Medicine, Tel Aviv University, Israel

Introduction: Ovarian hyperstimulation syndrome (OHSS) is characterized by hyperpermeability of the vascular bed, third space accumulation of fluid and plasma hyperconcentration, that is caused by ovarian stimulation. The therapeutic approach is to use plasma expanders and hence to prevent intravascular dehydration and oliguria. The aim of the present study was to evaluate the efficacy of a new plasma substitute, hydroxyethyl starch (HES), in the treatment of mild to moderate OHSS.

Materials and methods: In this retrospective controlled trial, the effect of HES solution was compared with that of Hemacell. The study and control groups comprised 20 women diagnosed with mild or moderate OHSS who had been admitted to the gynaecology department during a 12-month period. The two groups were matched for age, weight, degree of OHSS and existence of pregnancy. Treatment consisted of fluid intake, intravenous normal saline and intravenous plasma substitute, HES or Hemacell. Hemacell was used as plasma substitute to treat 10 women for the first 6 months, after which HES was used. Outcome measures were reduction of haematocrit, increase in urinary volume, weight loss and duration of hospitalization. The price of 1 unit of HES or Hemacell is

US$19.7 and US$6.8 respectively. Results were expressed as mean ± SE; significant differences were evaluated using a non-paired *t*-test.

Results: Women treated with HES or Hemacell received the same volume of fluid intake, intravenous normal saline and 2400 ± 748 ml HES or 1792 ± 540 ml Hemacell respectively (P = NS). Following treatment, the mean reductions in haematocrit in HES- and Hemacell-treated women were 5.5 ± 1.5% and 6.7 ± 1.7% respectively (P = NS). The mean increase in 24-h urine production in HES-treated women was 1345 ± 273 ml; this was not significantly different from the reduction following Hemacell treatment (1373 ± 168 ml). Both groups were hospitalized for 4.1 ± 0.7 days. The mean cost per treatment of a single woman with HES or Hemacell was US$94.56 and US$24.37 respectively.

Conclusion: HES is as effective as Hemacell solution in treatment of mild to moderate OHSS. However, Hemacell treatment was seen to be superior in terms of cost-effectiveness.

P-077. Correlation between day 2 embryo quality and blastocyst morphology

Paulmyer-Lacroix O., Charpenel L., Saias-Magnan J., Noizet A. and Grillo J.M.

Biologie de la Reproduction-CPMA, CHU la Conception, 147 Bd Baille, 13005 Marseilles, France

Introduction: The aim of this study was to relate day 2 embryo quality to blastocyst development and morphology.

Material and methods: After embryo transfer on day 2, 268 supernumerary diploid embryos were individually placed in extended culture (Ferticult or IVF-20 from day 0 to day 3;G2.2 from day 3 to day 5/6) for cryopreservation at the blastocyst stage. Embryo development was observed daily until day 5/6. Blastocysts were scored in three grades: grade A, good quality, with a large number of cells forming a well-defined and organized inner cell mass (ICM); grade B, with a large number of cells forming an unorganized ICM; and grade C, poor quality, with few cells forming the ICM. The relationship between blastocyst formation and morphology and day 2 embryo scoring (number and size of blastomeres, degree of fragmentation) was studied retrospectively. Statistical analysis was performed using a χ^2 test; $P < 0.05$ was considered significant.

Results: Seventy-four (27.6%) supernumerary embryos reached the blastocyst stage.

1. Relationship between day 2 embryo quality and blastocyst formation:

Day 2	No. of blastomeres		Size of blastomeres		Fragmentation (%)			
Day5/6	4	≠4	Equal	Unequal	0	1–20	20–50	>50
Blastocyst formation/ embryo in extended culture (%)	36.4 (40/110)	21.5 (34/158)	31.8 (44/138)	23.07 (30/130)	27.1 (16/59)	28.3 (39/138)	32.7 (16/49)	13.6 (3/22)
	$P < 0.05$		$P > 0.05$			$P > 0.05$		

2. Relationship between day 2 embryo quality and blastocyst morphology: The following data were obtained for blastocysts: 35.2% (26/74) were grade A, 24.3% (18/74) were grade B, and 40.5% (30/74) were grade C. Grade A and C blastocysts were derived in similar proportions from day 2, 4-cell embryos [61.5% (16/26) and 56.6% (17/30) respectively; P = NS] or having none or <20% fragments [80.8% (21/26) and 66.6% (20/30) respectively; P = 0.10]. Grade A blastocysts were derived more frequently from day 2 embryos showing equal-sized blastomeres than grade C blastocysts [73.1% (19/26) versus 40% (12/30); $P < 0.05$].

Conclusion: Our data show that on day 2: 4-cell embryos result in a higher percentage of blastocyst formation than ≠4-cell embryos; equal-sized blastomeres result in a higher percentage of good grade blastocysts (A) than unequal-sized cell embryos; no (or few) fragmentations have a tendency to give good grade blastocysts.

This suggests that the 4-cell stage on day 2 can be a predictive criterion to blastocyst formation, whereas blastomere size (and fragmentation degree) can be related to blastocyst morphology. This may be helpful when selecting embryos for transfer on day 2.

P-078. Rescue ICSI of 1-day-old unfertilized oocytes

Thonon F.[1], Jouan C.[1], Gaspard O.[1], Jamar M.[2], Herens C.[2], Godin P.A.[1], Hincourt N.[1], Foidart J-M.[1] and Dubois M.[1]

[1]Centre de Procréation Médicalement Assistée and [2]Center of Human Genetics, University of Liège, Belgium

Introduction: Total or near-total fertilization failure may occur in conventional IVF procedures for unexpected reasons, related to hormonal cycle characteristics or to spermatozoon–egg fusion defects. Several methods to circumvent this lack of fertilization were attempted on 1-day-old unfertilized oocytes: repeated insemination, partial zona dissection, subzonal insertion and intracytoplasmic sperm injection (ICSI; also called rescue ICSI). We describe here our clinical experience of rescue ICSI.

Materials and methods: When total or near-total failure (<30% fertilization) was observed, non-fertilized metaphase II (MII) oocytes were microinjected 24 h after retrieval with the spermatozoa used for the original insemination and conserved in fertilization medium at 37°C. On day 2 after retrieval, normally fertilized oocytes were cultured in G1-2 (Vitrolife, Sweden) for 24 h and transferred or frozen. In case of near-total fertilization failure with more than one conventionally fertilized embryos (CFE), rescue ICSI embryos (RIE) were frozen and CFE embryos preferably transferred. In two cases, combined transfer CFE-RIE were performed.

Results: Twelve rescue ICSI (12% of 97 IVF cycles) were performed. Altogether, 63 oocytes were fertilized. The fertilization rate was 65–68%, a value similar to that obtained in our conventional IVF procedure (66%). Among the eight women with total IVF failure, six transfers were performed, resulting in one pregnancy. In the two other women, development failure and lack of consent precluded the subsequent transfers. Among the four women with near-total failure, two combined transfers (CFE-RIE) resulted in one pregnancy. In both pregnancies, a high number of oocytes (20 and 12) were microinjected, and three embryos were transferred. In case 1 (pure RIE transfer), the fetus aborted at week 10 (normal karyotype 46,XX). In case 2 (transfer of two rescue ICSI embryos and one conventionally fertilized embryo), one fetus was aborted at week 8 (unknown karyotype) while the other is still alive at week 32 (normal karyotype 46, XY).

	Total failure	Near-total failure
No. of cycles	8	4
No. of MII injected oocytes (range/woman)	60 (1–20)	32 (5–12)
Rescue ICSI fertilization rate (2 PN)	65%	68%
No. of transfers	6 (pure RIE)	2 (RIE-CFE)
No. of pregnancies	1 (17%)	1 (50%)

Conclusion: Despite the limited number of procedures which preclude statistical validation, these preliminary data suggest that rescue ICSI can be offered to patients showing a high number of non-fertilized oocytes, in order to increase the probability to have good embryos for transfer. Based on the theoretical risk of cytogenetic abnormalities, antenatal diagnosis is even more advisable for such patients.

P-079. Assessment of oocyte maturity in intact cumulus–oocyte complexes

Godin P.-A., Gaspard O., Thonon F., Dubois M., Jouan C., Hincourt N. and Foidart J.-M.

Centre de Procréation Médicalement Assistée, University of Liège, Belgium

Introduction: In an IVF programme, in-vitro maturation (IVM) of germinal vesicle (GV) oocytes offers several advantages. The first is that ~15% of the oocytes are immature at the time of retrieval. IVM and subsequent fertilization may therefore result in an increased number of embryos available for transfer. Second, in some patients, the majority of oocytes are arrested at the GV stage. In such circumstances, IVM is the only opportunity to obtain embryos. Third, an efficient IVM procedure

could reduce ovarian overstimulation, its side effects and costs. Determination of oocyte maturity requires the removal of cumulus cells, but as these play an important role in oocyte maturation, it is critical to evaluate the oocyte status without separation of these cells. In this study, we describe an efficient technique to determine oocyte maturity in intact cumulus–oocyte complexes (COC).

Materials and methods: A total of 262 oocytes was collected from 18 patients undergoing IVF treatment. Before denuding the oocytes for intracytoplasmic sperm injection (ICSI), the COC were observed in a Petri dish containing HEPES-supplemented fertilization medium, using a dissecting microscope. Six oocytes were treated simultaneously. Gentle tilting of the dish (20° from horizontal) allowed the spreading and flattening of the cumulus. The ooplasm appeared clear and the GV (brown and crescent-shaped) was easily observed when present. Oocytes were divided into four groups: GV; metaphase I/II (no or one polar body); undetermined (often due to compact cumulus); or dead (fractured or empty zona pellucida (ZP), lysed oocyte).

Results: The technique was evaluated after removal of cumulus cells in 262 ICSI procedures. When maturity could not be determined within 15 s, oocyte was classified as undetermined (4.6%). For the detection of oocytes at the GV stage, efficiency was 89.6% and for metaphase I or II oocytes, it was 96%. Loss of oocytes was due to the ZP being damaged during denudation.

Estimation	Verification after cumulus cells removal				
	GV	Meta I[b]	Meta II[c]	Dead	Lost
29 GV[a]	26	1	2	0	0
212 meta I[b] or II[c]	8	9	185	1	9
12 undetermined	5	3	4	0	0
9 dead	0	0	0	9	0

[a]Germinal vesicle; [b]Metaphase I (no polar body); [c]Metaphase II (polar body I).

Conclusion: This simple technique of oocyte maturity determination is highly efficient. In avoiding denudation, it allows IVM of entire immature COC. The cumulus cells secrete a wide variety of regulatory growth factors, cytokines and chemokines. In addition, most endocrine factors do not directly affect the oocyte but exert an indirect action via cumulus cells. In consequence, such cell–cell interactions are very important for good-quality IVM. We believe that this procedure will facilitate IVM in current IVF procedures.

P-080. Relationship between free amino acid concentrations and IVF and pregnancy outcome

Strehler E.[1], Jelinkova L.[1], Knecht C.[1], Jöchle W.[2], Grünert A.[2] and Sterzik K.[1]

[1]Christian Lauritzen Institut, Frauenstraβe 56, 89073 Ulm, Germany and [2]Institute of Clinical Chemistry, University of Ulm, Germany

Introduction: The influence of amino acids on embryo development is still not clearly understood. The goal of this study was to evaluate the impact of the different amino acids in order to determine the relationship between these substances and IVF and pregnancy outcome. In our IVF collective, we compared the absolute concentration and relative patterns of 23 amino acids in blood plasma samples of patients with unsuccessful IVF cycles with those who became pregnant after IVF in regard to the pregnancy outcome.

Materials and methods: A total of 60 patients who underwent an IVF procedure and embryo transfer after gonadotrophin stimulation in down-regulated cycles, were recruited into the study. The reason for infertility was either a tubal or a male factor. Two to three embryos were transferred. The study group consisted of patients who did not become pregnant after IVF ($n = 6$), patients with pregnancy after IVF-embryo transfer ($n = 54$), but had a miscarriage until gestation week 12 ($n = 16$), as well as patients with ongoing pregnancies and live birth after IVF-embryo transfer ($n = 38$). The pregnant patients were further subdivided into two groups according to the number of repeated IVF attempts necessary to achieve pregnancy [group1: up to four IVF cycles ($n = 43$); group 2: more than four IVF cycles ($n = 11$)]. Blood samples were taken from each patient 2 weeks after embryo transfer. Quantitative and qualitative analysis of amino acids in blood was performed in all patients using liquid chromatography.

Results: Except for the relative proportion of the amino acid threonine, there were no significant differences noted between pregnant and non-pregnant patients. In the pregnancy group, the mean threonine amount was 5.40%, and in the non-pregnancy group 6.43% (Student's *t*-test, $P < 0.01$). In our study group the occurrence of miscarriage was associated with a significantly increased glutamate concentration (miscarriage group 45.31 µmol/l, ongoing pregnancy group 33.95 µmol/l). Furthermore, a relationship was shown between the relative and absolute arginine and citrulline concentrations and the total number of IVF attempts needed to achieve pregnancy [group 1: arginine 59.16 µmol/l (2.36%), citrulline 17.23 µmol/l (0.67%); group2: arginine 45.00 µmol/l (2.02%), citrulline 11.45 µmol/l (0.67%)]. The patient group with ⩾4 cycles to achieve a pregnancy had significantly higher arginine and citrulline concentrations (Student's *t*-test, $P < 0.05$). The absolute and relative concentrations of any other amino acid did not differ significantly between the different groups.

Conclusion: As yet, the effects of threonine and glutamate on embryo development in early pregnancy are not clearly understood. *In vivo*, L-arginine/citrulline serum concentrations are related to the nitric oxide (NO) system which is involved in the vasodilatory modulation of blood flow. Therefore, a possible influence of arginine and citrulline on vascular parameters and, in consequence, on IVF outcome may be assumed. Further investigations are necessary.

P-081. Immune cell subsets and interleukin concentrations in the peritoneal fluid of women with and without endometriosis

Gallinelli A., Ciaccio I., Salvatori M., Marsella T., Tirelli A., Lagalla C. and Volpe A.

Department of Gynecological, Obstetrics and Pediatric Sciences, University of Modena, Modena, Italy

Introduction: Recent studies have demonstrated a direct role of the immune system in the onset and development of pelvic endometriosis. Products of T lymphocytes such as cytokines seem able to modulate the ectopic growth of endometrium. Interleukin (IL)-12 and IL-13 are in fact considered to be prototypes of pro- and anti-inflammatory cytokines respectively. The present study explored possible correlations between immune cell subsets and IL-12 or IL-13 concentrations in the peritoneal fluid (PF) of patients with and without endometriosis.

Materials and methods: Our study group comprised 50 patients (aged between 27 and 43 years) with endometriosis at early stage (I and stage II, $n = 23$) or advanced stage (III and stage IV, $n = 27$) who were undergoing laparoscopy for either infertility or pelvic pain. The American Fertility Society (AFS) scoring system was used to determine the extent of endometriosis. PF concentrations of IL-12 and IL-13 were measured in the cell-free PF supernatants using an ultrasensitive commercially available ELISA kit, whereas PF mononuclear cells (PFMC) were cultured with phytohaemagglutinin and then analysed for immunophenotyping using a four-colour flow cytometry. Statistical analyses were carried out using the Mann–Whitney *U*-test and the χ^2-test. $P < 0.05$ was considered significant.

Results: Mean (± SD) concentrations of IL-12 in the PF of women with moderate or severe endometriosis (AFS score >10) were significantly higher than those in healthy controls (3.5 ± 0.7 and 1.9 ± 0.4 pg/ml respectively; $P < 0.01$), whereas no significant differences were observed between the two groups in case of minimal endometriosis (AFS score < 5). Subjects with endometriosis had significantly lower PF IL-13 concentrations than the healthy controls, independent of the grade of disease (89.7 ± 8.7 versus 113.1 ± 19.8 pg/ml). The concentrations of IL-12 and IL-13 in the PF of both patient groups did not show a menstrual cycle-dependent pattern. Moreover, the total number of $CD4^+$ T-cell subpopulation and the $CD4^+/CD8^+$ ratio were significantly higher in

patients with endometriosis than in healthy controls (ratio 2.05 ± 0.71 versus 1.28 ± 0.22%).

Conclusion: The present study has shown a relationship between PF IL-12 or IL-13 concentrations and the condition of endometriosis. Moreover, the concomitant variations observed in PF $CD4^+$ and $CD8^+$ subsets allow us to postulate that there is a reciprocal modulation between these cytokines and specific T-cell subpopulations.

P-082. Comparison of early or late follicular phase supplementation with gonadotrophins to achieve bifollicular development in normo-ovulatory women

Cedrin-Durnerin I., Galey J., Minot G., Uzan M. and Hugues J.N.

Center for Reproductive Medecine, Department of Gynaecology and Obstetrics, Jean Verdier Hospital, University Paris XIII, Bondy, 93143, France

Introduction: Evidence exists that increasing the number of pre-ovulatory follicles may be effective in improving the pregnancy rate in normo-ovulatory women with unexplained infertility, or in those undergoing intrauterine insemination for male infertility. However, while the outcome of cycles stimulated with FSH leading to more than one single follicle is significantly improved, the risks of multiple pregnancy and hyperstimulation are simultaneously increased. In most studies, the overall pregnancy rate tends to plateau when the number of follicles is more than two, but the risk of multiple pregnancy is strongly correlated with the degree of overstimulation. Therefore, a mild stimulation leading to a bifollicular development seems to be an optimal approach in these situations. However, it is still uncertain whether a slight but extended FSH stimulation would best be performed from the early to the mid follicular phase in order to surpass the FSH threshold, or from the mid to late follicular phase to prevent the physiological decrease in FSH concentrations. The aim of this prospective randomized study was to compare these two approaches.

Methods: Twenty-seven normo-ovulatory women were prospectively randomized to receive exogenous FSH injection either from day 2 to day 6 (group A), or from day 7 to day 11 of the cycle (group B). In each group, 112.5 IU of recombinant-FSH (Gonal F; Serono, Boulogne, France) was injected s.c. every day. On days 2, 7 and 12 of the cycle, blood samples were taken for measurement of hormone concentrations, and ultrasound (US) assessment of follicular development was performed.

Results: On day 2, both groups were similar with regard to hormonal and US evaluations. By contrast, on day 7, plasma oestradiol concentrations (mean ± SD) were significantly higher in group A than in group B (257 ± 137 versus 84 ± 50 pg/ml; $P < 0.005$). Moreover, the number of medium-sized (10–13 mm) follicles and the follicular growth of the larger follicle from day 2 to day 7 were higher in group A as compared with group B (2.8 ± 1.9 versus 0.8 ± 0.8 mm; $P = 0.002$; 6.6 ± 1.5 versus 2.6 ± 2.8 mm; $P = 0.001$ respectively). On day 12, plasma oestradiol concentrations were significantly lower in group A than in group B (281 ± 199 versus 512 ± 258 pg/ml respectively; $P = 0.01$). While the number of mature follicles (⩾14 mm) was not significantly different between groups (1.7 ± 0.9 versus 2.2 ± 1.9), follicular growth of the leading follicle between day 7 and day 12 was slower in group A than in group B (3.8 ± 1.8 versus 7.7 ± 3.8 mm; $P = 0.002$).

Conclusion: These preliminary data show that starting FSH stimulation at the early or late follicular phase of normo-ovulatory cycle is equally effective in achieving multiple development of medium-sized follicles. However, a lack of FSH supplementation in the late follicular phase seems to be detrimental for final follicular maturation. Hence, these results suggest that preventing the closure of the FSH window is more effective for the creation of a mild overstimulation than surpassing the FSH threshold.

P-083. Comparison of two depot gonadotrophin-releasing hormone analogues in an IVF programme: a prospective randomized study

Orvieto R., Krissi H., Kerner R., Ashkenazi J., Voliovitch I., Shelef M., Schwartz A., Ben-Rafael Z. and Bar-Hava I.

Department of Obstetrics and Gynecology, Rabin Medical Center, Petah Tiqva, and the Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel

Introduction: Gonadotrophin-releasing hormone analogues are widely used for pituitary suppression before gonadotrophin administration in IVF cycles. We compared the use of two depot gonadotrophin-releasing hormone analogues, leuprolide and triptorelin, in long suppressive ovarian stimulation protocols.

Material and methods: Patients were prospectively randomized to receive 3.75 mg depot formulations of either leuprolide or triptorelin on days 21–23 of the cycle. Stimulation with gonadotrophins was initiated after pituitary desensitization was achieved. The stimulation pattern and cycle outcome were compared between the groups.

Results: Twenty-six patients were included in each group. No statistically significant differences were observed in patient age (28.8 ± 4.1 versus 28.5 ± 4.7 years) or concentrations of oestrogen (6914 ± 2069 versus 6682 ± 3040 pmol/l) and progesterone (4.3 ± 2.46 versus 4.3 ± 1.95 nmol/l) on the day of human chorionic gonadotrophin (HCG) administration, gonadotrophin dosage (26.5 ± 8.6 versus 25.6 ± 6.8 ampoules), number of oocytes retrieved (16.4 ± 6.7 versus 18.6 ± 9.3), fertilization rate (0.51 ± 0.31 versus 0.45 ± 0.30), and number of embryos transferred (2.5 ± 1.3 versus 2.4 ± 1.4). However, statistically significant higher implantation and clinical pregnancy rates were found in the leuprolide group compared to the triptorelin group (26 versus 9% and 46 versus 19% respectively; $P < 0.05$).

Conclusion: Leuprolide as a depot preparation is associated with higher implantation and pregnancy rates than triptorelin when used in the mid-luteal phase as part of a long suppressive gonadotrophin-releasing hormone analogues protocol in IVF.

P-084. The outcome of assisted reproduction treatment cycles using urinary compared to recombinant gonadotrophins

Sharma V.[1], Salha O.[1], Dada T[1]. and Allgar V.[2]

[1]Assisted Conception Unit and [2]Department of Statistics, St James's University Hospital, Leeds, UK

Introduction: Previous studies have shown that the use of recombinant FSH (rFSH) for ovarian stimulation may be associated with a better outcome than urinary FSH or human menopausal gonadotrophin (HMG), probably due to the higher FSH bioactivity or the absence of LH. Very few studies have compared the efficacy of urinary HMG, FSH and recombinant FSH in the outcome of consecutive IVF and intracytoplasmic sperm injection (ICSI) cycles.

Materials and methods: Couples having their first attempt at the Assisted Conception Unit, St. James's University Hospital, Leeds, between April 1992 and March 2000 were counselled regarding the type of gonadotrophin to be used after considering the cost of the available preparations and their route of administration. The dose of gonadotrophin prescribed was decided as per a local standardized protocol dependent on the patients' age and body mass index.

Results: In total 2288 IVF and 754 ICSI cycles were analysed according to the type of gonadotrophin used (Table I). There was no significant difference between groups with regard to the patients' age, or the diagnosis and type of gonadotrophin received. Patients receiving rFSH needed fewer ampoules and a shorter duration of stimulation than those receiving HMG or FSH. Nevertheless, there was no difference between the groups with regard to number of oocytes retrieved, fertilized, cleaved, frozen or transferred. Although there was no statistical difference in the clinical pregnancy rate/cycle between groups, those using urinary FSH and HMG were 1.44 times and 1.71 times respectively more likely to become pregnant compared with patients receiving rFSH. Furthermore, the live birth rate/cycle in the urinary FSH and HMG groups was significantly

higher than that in the rFSH group. With regard to ICSI cycles, patients receiving HMG were significantly more likely to become pregnant compared with those receiving urinary FSH and rFSH.

Table I. Comparison of cycle outcome for the three groups

IVF cycles (n = 2288)	Urinary FSH	HMG	rFSH
Mean no. of ampoules used	39.9[a]	41.8[a]	36.5[a]
Duration of stimulation (days)	9.6[b]	10.6[b]	10.1[b]
Mean follicle diameter on day 8 (mm)	17.5	19.2	16.7
No. of cycles	1752	421	115
No. of patients pregnant	633	166	33
Clinical pregnancy rate/cycle (%)	36.1	39.4	28.7
Live births	450	146	19
Live birth rate/cycle (%)	25.7[c]	27.6[c]	16.5[c]
ICSI cycles (n = 754)			
No. of cycles	102	616	33
No. of patients pregnant	29	215	6
Clinical pregnancy rate/cycle (%)	28.4[d]	34.9[d]	18.2[d]
Live births	17	143	6
Live birth rate/cycle (%)	16.7	23.2	18.2

[a], [b], [c], [d]Statistically significant ($P < 0.05$) differences.

Conclusion: These data reflect the outcome of routine clinical practice over 8 years, and suggest that higher pregnancy and live birth rates can be achieved with urinary preparations for both IVF and ICSI cycles. These clinically derived retrospective data are in agreement with results obtained in recently published prospective randomized trial.

P-085. ICSI with cryopreserved testicular or epididymal spermatozoa: a comparative study

Grizard G., Force A., Hermabessiere J., Pouly J.L., Janny L. and Boucher D.

Biologie du Développement et de la Reproduction-CECOS-FIV; CHU 63003 Clermont-Ferrand, France

Introduction: Testicular and epididymal spermatozoa are used routinely for intracytoplasmic sperm injection (ICSI) to treat men with azoospermia. In combination with ICSI, cryopreservation of such spermatozoa is of benefit to the couple. Indeed, it avoids the need for repetitive biopsies for successive ICSI cycles, and unnecessary ovarian stimulation of the female partner in case of sperm non-availability. It seems, however, that epididymal spermatozoa can be frozen more easily because of their higher concentration and motility compared with testicular spermatozoa. The aim of the study was to compare the outcome of ICSI with frozen–thawed testicular or epididymal spermatozoa.

Materials and methods: Between February 1996 and July 2000, 52 ICSI cycles with epididymal spermatozoa and 21 ICSI cycles with testicular spermatozoa were carried out. Spermatozoa were collected by microepididymal sperm aspiration (MESA) in 26 men with obstructive azoospermia and by testicular biopsy (TESE) in 13 men (seven with obstructive azoospermia, six with non-obstructive azoospermia). The tissue was processed immediately after retrieval. After centrifugation, the cell suspensions were diluted dropwise (v/v) with the cryoprotective medium (final concentration of glycerol 7%, v/v), maintained for 15 min at room temperature, and finally sealed in 0.25 ml straws. Cooling and freezing between storage in liquid nitrogen were carried out under computer control. On the day of oocyte puncture, samples were thawed (3 min at 37°C), progressively diluted, centrifuged and selected on gradient density (Puresperm®).

Results: The quantity of motile epididymal spermatozoa recovered after freezing–thawing and selection on gradient density was very variable among the patients, but it was better than that of testicular spermatozoa for which overt motility with poor forward motility was rarely observed. Except in one case with epididymal spermatozoa, cleaved embryos were obtained in all ICSI cycles. No significant differences were noted between ICSI with testicular or epididymal spermatozoa: mean injected oocytes per cycle 8.5 ± 1.2 versus 9.7 ± 0.8; cleaved embryos per cycle 4.8 ± 0.8 versus 5.9 ± 0.5; transferred embryos per cycle 2.3 ± 0.2 versus 2.5 ± 0.1; clinical pregnancy per embryo transfer 19.0 versus 23.5%; implantation rate 14.6 versus 14.5%; and delivery/ongoing pregnancy 14.2 versus 13.1%. In order to see if no selection was induced by several ICSI, calculations were performed by taking into account only the first ICSI cycle, and for all the parameters considered, no difference was found between ICSI with testicular or epididymal spermatozoa.

Conclusion: Frozen–thawed testicular or epididymal spermatozoa yield similar cleavage and ongoing pregnancy rates using ICSI. Consequently, cryopreservation of testicular spermatozoa may be recommended before ICSI.

P-086. Efficacy of double (at 12 and 36 h) versus single (36 h) endouterine insemination

Garijo E., García J.L., Villafáñez V., Verdú V., Bajo J.M. and Galera F.

GINEFIV, Madrid, Spain

Introduction: Pregnancy rates obtained after endouterine insemination, although still lower than those achieved after IVF, could be improved by performing more than one insemination procedure during the periovulatory period. The objective of this study was to analyse pregnancy rates in patients undergoing single insemination at 36 h after the human chorionic gonadotrophin (HCG) peak, and comparing outcome with patients receiving double insemination at 12 and 36 h after the same peak.

Materials and methods: A pilot study was carried out in 28 patients with no known history of gynaecological disease, age <40 years, and male factor with MER $>5\times10^6$. All underwent double insemination (at 12 and 36 h). All pregnancy data obtained from these patients was compared with our existing database for similar patients receiving single insemination at 36 h. All data were analysed using the SPSS 9.0 package and the χ^2 test.

Results: Results are listed in the tables.

		12–36 h	36 h	P
Pregnancy	IA	35.7%	20.65%	NS
IAH	19%	16.36%	NS	
	IAD	85.7%	27.03%	NS

Follicles	12–36 h	36 h
Pregnancy	2.7	2.15
No pregnancy	1.15	1.94
	$P < 0.05$	$P < 0.01$

	12–36 h	36 h	P
1° cycle	18.2%	22.22%	NS
2° cycle	40%	17.39%	NS
3° cycle	100%	25%	NS
4° cycle	66%	40%	NS

Conclusion: Although these data are preliminary in nature, double insemination appears to result in higher pregnancy rates than single insemination, and with a lower number of cycles. However, these data should be confirmed in future studies using a greater number of patients and a correct comparative, randomized analysis.

P-087. Comparison of the cumulated deliveries obtained after transfer of three versus two embryos when freezing the supernumerary embryos at the zygote stage

Senn A., Primi M.-P., Chanson A., Wirthner D., De Grandi P. and Germond M.

Reproductive Medicine Unit, Department of Obstetrics and Gynaecology, CHUV CH-1011 Lausanne, Switzerland

Introduction: The IVF success rate is dependent mostly on the quantity and quality of transferred embryos, and the occurrence of multiple

gestations has been considered as an inevitable by-product of the technique. Transfer of fewer embryos, cryopreservation and in-vitro selection of blastocysts provide theoretical means of reducing multiple pregnancies. However, freezing of all supernumerary embryos at the pronuclear stage does not allow embryo selection to be made on either morphological or developmental bases. A reduction in the number of transferred embryos from three to two under a strict zygote cryopreservation policy may thus lead to confounding and deceptive results, if the cumulated pregnancies originating from fresh and cryopreserved cycles are not considered. The purpose of this study was to compare the cumulated pregnancy and delivery rates, by analysing retrospectively the outcome of two transfer policies involving either three or two embryos.

Materials and methods: Fresh (n =2542) and frozen–thawed (n = 1945) transfers originating from oocyte collections performed between January 1992 and December 1999 were considered. Cycles in which embryos were cryopreserved at cleaved stages were excluded. Fresh embryo transfers were performed on day 2, at 48–52 h after oocyte retrieval. Embryos were cryopreserved at the pronuclear stage using the slow protocol and a HEPES-buffered medium in the presence of sucrose (0.1 mol/l), propanediol (1.5 mol/l) and synthetic serum substitute (10%; Irvine Scientific, CA, USA). Zygotes were thawed 24–28 h before transfer, 3 days after the LH peak in natural cycles. All cryopreserved cycles were related to the original IVF cycles; the cumulated pregnancy and delivery rates were analysed with respect to parameters of the fresh IVF cycle, such as maternal age, number of available zygotes on day 1 and current transfer and freezing policies (three versus two).

Results: The cumulated pregnancy rate was dependent on the number of zygotes available on day 1. The cryopreserved zygotes contributed significantly to the final pregnancy rate when more than five zygotes were obtained. The cumulated pregnancy rate reached a value close to 70% when ⩾11 zygotes were available on day 1. Whereas triple pregnancies were almost completely absent (0.3%) under the two-embryo transfer policy, the incidence of twin gestation was similar in both groups (Table I). When three or more zygotes were available on day 1, the cumulated delivery rates achieved under a three- or two-embryo transfer policy was not significantly different for all age groups (Table II).

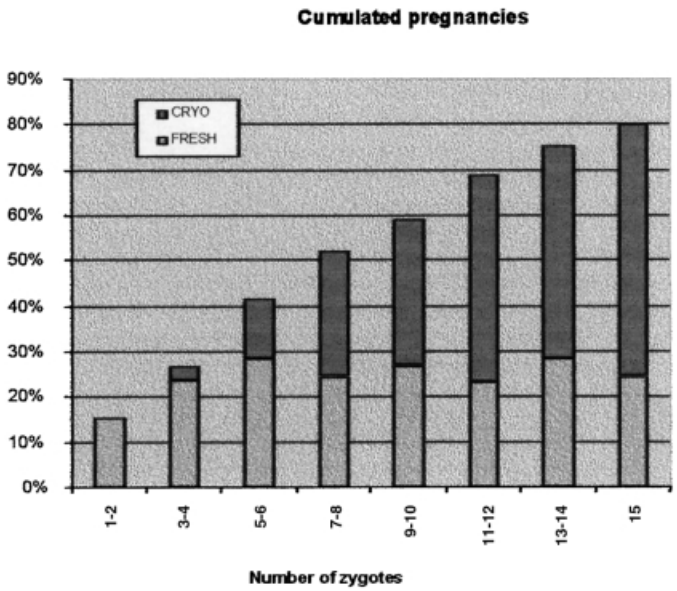


Table I. Multiple pregnancy rates

No. of sacs	3 embryos	2 embryos	*P*
1	271 (72.7%)	260 (79.0%)	0.05
2	79 (21.2%)	68 (20.7%)	NS
3	22 (5.9%)	1 (0.3%)	0.0001
4	1 (0.3%)	0 (0.0%)	NS

Table II. Cumulated delivery rates

Age (years)	3 embryos	2 embryos	*P*
<35	130/348 (37.4%)	199/479 (41.5%)	NS
35–39	96/343 (28.0%)	60/195 (30.8%)	NS
⩾40	19/109 (17.4%)	9/28 (32.1%)	NS
Total	245/800 (30.6%)	268/702 (38.2%)	0.002

Conclusion: When cryopreservation was performed at the pronuclear stage, reducing the number of transferred embryos from three to two was not associated with any decrease in the cumulated delivery rate. Whereas as triple gestations were significantly reduced, the incidence of cumulated twin pregnancies remained unchanged.

P-088. White cell galaxy (leukocytospermia) and antioxidant supplement therapy in the primary infertility group of normozoospermic young patients

Atassi M.E.[1], Idriss W.K.[2] and Sambasivarao K.[2]

[1]Arabic Center Hospital, P. O. Box 4138, Homs, Syria and [2]Arab Fertility Center, Khalid Idriss Hospital, P. O. Box 75, Jeddah, Saudi Arabia

Introduction: Microbial infection of accessory sex glands is one of the important causes of infertility in young men according to WHO reports. The inflammatory process resulting from infection leads to increased leukocyte count in semen samples. The fertility-inhibiting effect of leukocytospermia ('white cell galaxy') is treatment-amenable, particularly in normozoospermic young patients. The aim of the present study was to identify the therapeutic value of antioxidant supplement therapy in white cell galaxy.

Materials and methods: Thirty-one male patients (age 22–34 years) of a primary infertility group, who attended our fertility centres during the year 2000, were diagnosed with white cell galaxy. Routine gynaecological examinations and laboratory tests did not reveal any pathological abnormalities in the wives. Similarly, the husbands were also subjected to the basic infertility screening procedures and found normal. However, the semen analysis reports showed evidence of white cell galaxy in all the patients, though the sperm concentration and other semen parameters were within the normal range of values as per WHO standards. The technique of immunohistological characterization and quantification of leukocytes was followed in this method. All patients were treated initially with the following combinations of drug therapy: rifampicin 600 mg/day, cephalexin 500 mg/day, vitamin C 1000 mg/day and acetylsalicylic acid 100 mg/day. The patients were advised to discontinue the first two antibiotics after 3 weeks of treatment, and asked to continue the other two drugs for a further 2 months. The complete semen analysis was repeated again after 3 months of initial treatment. The status of white cell galaxy was then assessed. The patients, who showed only partial recovery, were advised to repeat the same treatment once again for a further 3-week period. Those patients who showed no improvement and still had a persistent leukocyte count were advised to follow another treatment schedule of: ciprofloxacin 500 mg/day for 4 weeks along with vitamin C and acetylsalicylic acid in the usual dosage schedule.

Results: Twenty-five patients showed a clear improvement, with a normal round cell count as per WHO standards, whilst the other six patients showed sporadic appearance of leukocytes confined to only few high-power microscopic fields. Surprisingly, the wives of six patients became pregnant by natural means without assisted reproductive technology procedures, and four patients achieved pregnancy by intrauterine insemination (IUI) procedures. The other 12 patients were still undergoing repeated attempts at IUI treatment.

Conclusion: White cell galaxy (leukocytospermia), which is one of the inhibiting factors of the fertilizing ability of spermatozoa, is amenable to drug treatment with antioxidant supplement. The results are compared and discussed.

P-089. Selection of spermatozoa with low frequency of chromosomal aneuploidies: preparation by swim-up versus gradient centrifugation

Kovacs T., Kovanci E., Vigue L., Corrales M. and Huszar G.

The Sperm Physiology Laboratory, Yale University School of Medicine, New Haven, CT, USA

Introduction: We have found that upon 40/80% Percoll gradient fractionation of semen, the sperm chromosomal aneuploidies and the proportion of immature spermatozoa with cytoplasmic retention, which reflects incomplete spermiogenetic maturation, has declined in the 80% pellet. In the present study, we have examined the efficiency of swim-up preparation in eliminating spermatozoa with chromosomal disomies and diploidies. Although similar experiments have been performed by other laboratories, the data are controversial, because the numbers of sperm nuclei evaluated were not sufficient to develop conclusive results in light of the low aneuploidy rates in ejaculated human spermatozoa.

Materials and methods: To date, we have studied six men [mean ($\pm$ SEM) sperm concentration 23.1 $\pm$ 5.6$\times 10^6$ spermatozoa/ml]. For the fluorescence in-situ hybridization (FISH) studies, sperm smears of initial semen were prepared and fixed with methanol-acetic acid. Another semen aliquot was diluted with human tubal fluid (Irwin Scientific Co.) and centrifuged at 600$\times g$ for 10 min in a flat-bottomed tube. Subsequently, the supernatant with the exception of 0.5 ml was removed, and a swim-up step was carried out for 25 min at 36°C. Motility in the initial and swim-up sperm fractions was 43.3 $\pm$ 2.8% and 75.6 $\pm$ 9.6% respectively ($P < 0.001$). The yield of motile spermatozoa in the swim-up fraction compared with initial semen was 46.2 $\pm$ 8.1%. An aliquot of the swim-up spermatozoa was also fixed to glass slides, and both the semen and swim-up samples were subjected to FISH, using centromeric probes for the X, Y, 10, 11 and 17 chromosomes. The incidences of immature spermatozoa were determined by creatine phosphokinase (CK)-immunocytochemistry, which highlights cytoplasmic retention. Data analysis was carried out using χ^2 analysis and the paired *t*-test.

Results: Data from 125 000 spermatozoa examined, in the six initial semen and swim-up sperm fractions, indicated that there were no changes in the X/Y ratios. With regard to sperm nuclei with X, Y or XY sex chromosome disomies, or the combined data of the three autosomal chromosomes (10, 11, 17) there were no changes in disomy frequencies. Chromosome 17 disomies alone showed a decline ($P = 0.007$) in the swim-up versus initial semen fractions. Also, there was a substantial decline in the frequency of diploidies in swim-up spermatozoa ($P < 0.001$), similar to the decline in the incidence of immature spermatozoa with cytoplasmic retention ($P < 0.001$) in the swim-up versus initial semen.

Sperm scored	X/Y ratio	X+Y+XY disomy (%)	10+11+17 disomy (%)	17 disomy (%)	All disomies (%)	Diploidy (%)	Immature spermatozoa (%)	
Semen	63 465	1.065	0.21	0.45	0.12	0.66	0.49	41.6
Swim-up	62 168	1.040	0.16	0.29	0.06	0.45	0.19	30.6
P		NS	NS	NS	0.017	NS	< 0.001	< 0.001

Conclusion: In comparing the efficiency of sperm preparation methods for elimination of immature spermatozoa and spermatozoa with chromosomal aberrations, we found that swim-up is less efficient than gradient centrifugation in the selection of mature spermatozoa with low frequency of aneuploidies. After swim-up fractionation, the diploidy frequency and the proportion of immature spermatozoa were lower in the swim-up fraction. However, among disomies, only the incidence of 17 disomy has declined, whereas in the 80% Percoll pellet all disomies studied showed lower frequencies ($P < 0.01$ to 0.001). Thus, discontinuous gradients are more efficient than swim-up fractionation in sperm preparation for assisted reproduction.

P-090. Intratesticular Doppler flow in fertile and infertile males

Rossi S.[1], Regnani G.[2], Mancini F.[1], Giulini S.[2], Battaglia C.[1], Pollastri P.[1] and Venturoli S.[1]

[1]Reproductive Medicine Unit, S. Orsola Hospital, University of Bologna, Via Massarenti 13, Bologna, Italy and [2]Department of Obstetrics and Gynecology, University of Modena, Via del Pozzo 71, Modena, Italy

Introduction: Ultrasonography and colour Doppler provide finding of tissue morphology, and also facilitate the detection of small intratesticular vessels in males undergoing assisted fertilization techniques. The aim of this study was to evaluate the modifications of testicular volume and pulsatility index (PI) in fertile and infertile males.

Materials and methods: Fifty-six males [mean ($\pm$ SD) age 37.6 $\pm$ 6.3 years] who were undergoing assisted reproduction were recruited after providing their informed consent. Subjects were allocated to three groups according to semen analysis: normozoospermic ($n = 16$); oligozoospermic (sperm concentration $<10\times 10^6$/ml) ($n = 21$); and azoospermic ($n = 19$). Patients were excluded if they were affected by: varicocele, diabetes, hypertension, testicular injury, previous testicular surgery and abnormalities of scrotal content. Smokers and patients receiving ongoing medical treatment were also excluded. All patients were submitted to ultrasonographic evaluations of testicular volume and PI of the transmediastinal artery (TMA). The PI reflects blood flow impedance downstream from the point of sampling.

Results: No significant differences were observed between the volumes of the left and the right testicles. The assessment of ultrasonographic volume showed significantly smaller testes in the azoospermic group than in oligozoospermic group (12.4 $\pm$ 3.1 versus 15.3 $\pm$ 4.3 ml; $P < 0.05$) and even greater size reduction in the normozoospermic patients (12.4 $\pm$ 3.1 versus 19.7 $\pm$ 4.1 ml; $P < 0.001$). No significant differences between the PI of the left and right TMAs were found. Higher resistances (PI) in the TMAs were observed between azoospermic and normozoospermic patients (1.47 $\pm$ 0.2 versus 1.19 $\pm$ 0.25; $P < 0.05$). Normozoospermic subjects, compared with oliogozoospermic, were characterized by higher, but not significant, resistances in the TMA (1.47 $\pm$ 0.2 versus 1.37 $\pm$ 0.21).

Conclusion: This study shows a positive correlation between sperm count and ultrasonographic testicular volume, as well as an increased resistance in the small vessels distal to the TMA in infertile males.

P-091. Gender selection of human spermatozoa: a simple and effective approach

Bartsich S., Hariprashad J.J., Rosenwaks Z. and Palermo G.D.

The Center for Reproductive Medicine and Infertility at the Weill Medical College of Cornell University, New York, NY, USA

Introduction: Gender preselection has enormous implications for both animal and human reproduction. While many studies have been performed to separate X- and Y-chromosome-bearing cells, very few of those claiming to be successful have proven to be repeatable. Cell sorting, the one effective technique, achieves a true enrichment of Y-bearing cells, but it requires a major equipment investment and raises additional concerns about sperm DNA related to fluorescent staining and UV excitation. In this study, we have used a static density gradient to select sex-specific human spermatozoa. In order to avoid sperm motility as a confounding factor in the density selection method, a partial immobilization of the spermatozoa was chemically performed.

Materials and methods: Samples which displayed normal parameters according to the WHO and Kruger's criteria were obtained by masturbation from five healthy consenting males undergoing routine semen analysis (mean age 38.0 $\pm$ 10 years, mean sperm concentration 111.4 $\pm$ 21$\times 10^6$/ml, mean sperm motility 56.8$\pm$7%). Specimens were washed in human tubal fluid (HTF) and their concentration was adjusted to ~100$\times 10^6$/ml. A six-layer density gradient was prepared by layering Percoll solutions in HEPES-HTF at 0, 20, 40, 60, 80 and 100% (v/v) in a Falcon 50 ml tube. Although spermatozoa were still flagellating, their motility was rendered non-progressive by chemical treatment with lysophosphatidyl-

choline (LPC; 5 ng/ml for 15 min). The cells were then washed by centrifugation (500×*g* for 15 min) and resuspended in HTF medium to achieve a concentration of ~5×10^6/ml. The resuspended sample was layered on top of the gradient, which was then incubated at 4°C for 90 min. Layers were carefully aspirated from the top. Resulting fractions from the top and bottom layers were centrifuged in HTF medium to remove residual silica gel particles, and the resulting pellets were resuspended and smeared on slides for fluorescence in-situ hybridization (FISH) analysis using centromeric probes for chromosomes 18, X, and Y (Vysis). The ratio of X- to Y-chromosome-bearing spermatozoa was blindly assessed as a percentage on at least 200 cells per slide. Aneuploid cells and those without signals were omitted. Untreated fractions of each sample served as controls.

Results: The untreated samples had a mean concentration of 51.3 ± 3% X-bearing cells, and 48.7 ± 3% Y-bearing cells ($P = 0.03$). Fractions collected from the top layer of the gradient revealed a 62.4 ± 2% concentration of Y-bearing spermatozoa versus 37.6 ± 2% of X-bearing cells ($P = 0.0001$), and those from the bottom revealed a concentration of 58.5 ± 1% of X-bearing cells versus 41.5 ± 1% Y-bearing cells ($P = 0.0001$).

Conclusion: While there was no difference in the ratio of X- and Y-chromosome-bearing cells in the untreated sample, there was a significant enrichment of Y-bearing cells in the top layer and X-bearing cells in the bottom layer after exposure to the Percoll gradient system. Therefore, this study represents the first step in the development of a simple, minimally invasive, and effective method of gender selection of human spermatozoa.

P-092. Clinical experience on in-vitro maturation of human oocytes

Suikkari A.-M. and Tuuri T.

The Infertility Clinic of The Family Federation of Finland, PO Box 849, 00101 Helsinki, Finland

Introduction: The development of means to mature human oocytes *in vitro* for IVF and ICSI will advance assisted reproduction towards a more patient-friendly and safer future. The complexity of cytoplasmic maturation of oocytes has been and still is the rate-limiting process in the development of in-vitro maturation (IVM) of human oocytes into a fully operational clinical practice. Nevertheless, progress has been made and consistent clinical success has been published. Several clinical protocols, with or without low-dose FSH in late luteal or early follicular phase, have been used to enhance the retrieval of developmentally competent immature oocytes. For the past 14 months we have used no FSH priming in a 5-day-week clinical programme, with encouraging results.

Patients and methods: Twenty-four patients with a previously determined need for IVF or ICSI were recruited. Sixteen women had regular menstrual cycles between 26 and 32 days and eight women had either minor or major irregularities. Three women had polycystic ovaries. The means ± SD age of the women was 31 ± 3.7 years (range 24–37), BMI 22 ± 3.1 kg/m^2, and duration of infertility 43 ± 28 months. The cycles were monitored by two ultrasound examinations on average. Oocyte retrieval (OPU) was scheduled when the leading follicle was approximately 10 mm in diameter or when the endometrial thickness was at least 6 mm. Oocytes were recovered by ultrasound-guided transvaginal aspiration of visible follicles as described previously. Maturation and fertilization of oocytes were performed as previously described, with the exception that maturation time was reduced to 36 h and patient's serum was used instead of fetal bovine serum. Oral oestradiol valerate and transvaginal micronized progesterone was administered after OPU to allow endometrial priming for embryo transfer. Embryos were transferred on day 2 or 3 after ICSI. Good quality supernumerary embryos were frozen.

Results: Twenty-five cycles resulted in 22 fresh embryo transfers and eight frozen embryo transfers. OPU was done on mean cycle day 10 (range 7–17). One patient was amenorrhoeic and had had a progesterone-induced bleed 45 days prior to OPU. Consequently she became pregnant. Endometrial thickness at OPU was 5.6 ± 1.1 mm (mean ± SD). The size of the largest follicle at OPU was 11 ± 1.9 mm (range 8–17 mm). Mean number of oocytes retrieved was eight (range 1–25). Oocytes were recovered in all patients. Maturation and fertilization rates were 70 and 76% respectively. One embryo was available for transfer in three fresh and two frozen cycles. In all other cycles, two embryos were transferred. Only three patients did not have transferable embryos. Extra embryos were frozen in six (24%) cycles. Five clinical pregnancies and one biochemical pregnancy resulted. One healthy baby girl has been born and four pregnancies are ongoing.

Conclusions: IVM together with ICSI seems a promising new assisted reproductive method which can be used for women with both regular and irregular cycles and PCO. The need for FSH priming and optimizing the timing for oocyte retrieval and maturation needs further research.

P-093. Timing of intrauterine insemination: the use of GnRH antagonist versus ovulation detection kit in FSH-stimulated cycles

Baroni E., Ragni G., Guermandi E., Riccaboni A., Arnoldi M., Scarduelli C. and Crosignani P.G.

Department of Obstetrics and Gynaecology, University of Milan, Milan, Italy

Introduction: There is no consensus in the literature on the optimal timing of intrauterine insemination (IUI), but most authors suggest that it is better to perform a periovulatory IUI. Among the different methods available, ultrasound monitoring of folliculogenesis and home urinary LH detection kits are the most widely used for ovulation prediction and detection. Recently, it has been pointed out that the use of gonadotrophin-releasing hormone (GnRH) antagonists in individuals undergoing ovarian stimulation prevents the premature luteinization and improves the outcome of assisted reproductive techniques. GnRH antagonists suppress gonadotrophin production by competitive inhibition of the binding of GnRH to its receptors on gonadotroph cell membranes. The aim of this prospective and randomized study was to compare the effectiveness of GnRH antagonist versus urinary LH detection kit for the timing of IUI in gonadotrophin-stimulated cycles.

Materials and methods: Forty-one couples with male or unexplained infertility underwent ovarian stimulation and IUI. Ovarian stimulation was carried out with recombinant-FSH, and patients were randomly assigned to one of the following groups: group A ($n = 19$) received GnRH antagonist when the ultrasound measurement of at least one follicle was ⩾14 mm; and group B ($n = 22$) underwent urinary LH assessment with the use of commercial kits (Clearplan, Farmades, Rome, Italy), starting when a follicle of ⩾16 mm mean diameter was measured. In group A, human chorionic gonadotrophin (HCG) was given when the leading follicle reached 20 mm in mean diameter, and IUI was performed 34 h later. In group B, HCG was administered when urinary LH was detected, and IUI was performed 6 to 18 h later. No luteal phase supplementation was given.

Results: Forty-eight cycles were completed in 41 patients. Six pregnancies occurred, for an overall pregnancy rate of 12.5% per cycle. The pregnancy rates with GnRH antagonist-timed IUI were 15.8% per patient (3/19) and 13.6% per cycle (3/21), and the pregnancy rates with LH-timed IUI were 13.6% per patient (3/22) and 11.1% per cycle (3/27). The differences in these rates between group A and group B were not statistically significant.

Conclusion: Our data indicate that both methods for timing IUI can be effective. The GnRH antagonist allows, albeit at high cost, a definite LH suppression and a better organization for the clinicians in that IUI can always be performed 34 h after HCG administration. Even though the home urinary LH detection kit has a lower cost and provides similar results, it implies a more difficult time plan for the IUI, since the timing itself depends on the variability of urinary test results.

P-094. Preovulatory follicles of women with polycystic ovarian syndrome express a specific cytokine profile during assisted reproduction cycles

Lédée-Bataille N.[1], Laprée-Delage G.[2], Taupin J.-L.[3], Dubanchet S.[2], Taieb J.[4], Fernandez H.[1], Hamamah S.[1], Moreau J.-F.[3], Frydman R.[1] and Chaouat G.[2]

[1]Department of Obstetrics-Gynecology and IVF Center, Antoine Béclère Hospital, Clamart, France, [2]INSERM U131, Clamart, France, [3]CNRS, UMR 5540, Bordeaux, France and [4]Department of Biochemistry, Antoine Béclère Hospital, Clamart, France

Introduction: In order to investigate whether a specific cytokine profile could be detected in the ovaries of patients with polycystic ovarian syndrome (PCOS), we collected follicular fluid (FF) from preovulatory follicles collected after ovarian stimulation from 15 PCOS patients, 15 infertile control patients with regular cycles, and eight oocyte donors

Materials and methods: ELISA or bioassays were used to assess the concentrations of leukaemia inhibitory factor (LIF), tumour necrosis factor (TNF), interleukin 11, gamma interferon, progesterone and oestradiol in FF.

Results: LIF and progesterone concentrations were significantly lower in the FF of PCOS patients (LIF median 265 pg/ml) compared with controls (LIF median 816 pg/ml); LIF and progesterone FF concentrations were correlated ($r = 0.720$, $P = 0.0001$). The LH/FSH ratio was negatively correlated with LIF concentrations ($r = –0.714$, $P = 0.0075$). Although the PCOS and control patients did not differ significantly as to age, ovarian reserve or IVF indication, the implantation rate was significantly lower among the women with PCOS (9% versus 21%).

Conclusion: The specific cytokine profile of PCOS patients is probably related to the lower implantation rate since in the follicular fluid, LIF appears to function as an embryotrophic agent. This relation raises the question of the supplementing culture media with LIF for PCOS patients undergoing IVF.

P-095. Utero-ovarian blood flow characteristics of pituitary desensitization: a prospective longitudinal study

Dada T., Salha O. and Sharma V.

Assisted Conception Unit, St. James's University Hospital, Leeds LS9 7TF, UK

Introduction: Currently, successful pituitary down-regulation is assessed by suppression of pituitary and ovarian hormones and/or measurement of endometrial thickness. In this report we studied the blood flow changes that occur at down-regulation after gonadotrophin-releasing hormone analogues (GnRHa) usage to establish whether colour flow Doppler can be used to monitor pituitary suppression, and whether these changes have a relationship with quantitative changes in pituitary and ovarian hormone levels.

Materials and methods: Patients with a regular menstrual cycle and a body mass index (BMI) <32 kg/m^2 undergoing assisted reproduction treatment (ART) were recruited. All patients had colour flow Doppler (CFD) velocimetry assessment of the utero-ovarian arteries $\leqslant 3$ days before the start of menses (baseline, BL) and after 21 days of GnRHa treatment (pre-stimulation, PS). At least four waveform patterns were analysed for calculation of the pulsatility index (PI), resistance index (RI), peak systolic velocity (PSV) and the time-averaged maximum velocity (TAMV). Ovarian volume, endometrial thickness, pituitary and ovarian hormone concentrations were also recorded.

Results: A total of 75 patients with a mean age of 33.2 years (range 28–42 years) were studied. Thirty-two patients had IVF treatment, 20 patients frozen–thawed embryo transfer cycles, and 23 patients were recipients for egg donation cycles. Significant changes were noted in utero-ovarian vasculature during the down-regulation period (Table I), as well as a significant reduction in ovarian and pituitary hormone concentrations, ovarian volume and endometrial thickness (Table II), with good correlation between RI and oestradiol estimations. Neither the type of GnRHa studied nor age influenced the noted changes in utero-ovarian blood flow. Ovarian artery RI was the best Doppler predictor for adequate pituitary suppression, and a cut-off value of 0.87 ± 0.025 was found to have the highest specificity and positive predictive value.

Table I. Comparison of baseline and pre-stimulation CFD characteristics in the ovarian and uterine arteries

	RI	PI	PSV (cm/s)	TAMX (cm/s)
Right ovarian artery				
Baseline	0.88 ± 0.1[a]	3.79 ± 1[a]	20.9 ± 6[a]	10.1 ± 3[a]
Pre-stimulation	0.93 ± 0.1[a]	4.27 ± 1[a]	15.8 ± 4[a]	6.7 ± 3[a]
Left ovarian artery				
Baseline	0.87 ± 0.1[b]	3.51 ± 1[b]	22.9 ± 8[b]	11.4 ± 6[b]
Pre-stimulation	0.93 ± 0.1[b]	4.36 ± 2[b]	17.4 ± 7[b]	7.71 ± 3[b]
Uterine artery (mean)				
Baseline	0.83 ± 0.1[c]	2.58 ± 1[c]	32 ± 10.9[c]	16 25 ± 5[c]
Pre-stimulation	0.89 ± 0.1[c]	3.34 ± 1[c]	24.6 ± 7[c]	10.5 ± 4[c]

Values are expressed as mean ± SD. [a], [b], [c] = significant difference ($P < 0.05$) between BL and PS.

Table II. Comparison of serum hormone concentrations and ovarian volumes at baseline and pre-stimulation.

	Baseline	Pre-stimulation	*P*
Oestradiol (pmol/l)	276.4 ± 197	68.6 ± 48	< 0.0001
FSH (U/l)	3.2 ± 2	2.5 ± 2	NS
LH (U/l)	6.5 ± 4.5	0.7 ± 1	< 0.0001
Progesterone (nmol/l)	21.2 ± 12	1.08 ± 1	< 0.0001
Right ovarian volume (cm^3)	9.3 ± 5	6.7 ± 3	< 0.001
Left ovarian volume (cm^3)	8.2 ± 5	6 ± 4	< 0.001
Endometrial thickness (mm)	10.5 ± 5.6	3.3 ± 0.2	< 0.0001

Values are expressed as mean ± SD. NS = no significant difference.

Conclusion: For the first time, we have identified cut-off values for satisfactory pituitary suppression in ovarian artery blood flow, with high specificity and positive predictive value.

P-096. Establishing a reliable assay for routine detection of Y microdeletions in different male patients undergoing ICSI

Takeshita N., Bartsich S., Neri Q.V., Spandorfer S.D., Rosenwaks Z. and Palermo G.D.

The Center for Reproductive Medicine and Infertility, Weill Medical College of Cornell University, New York, NY, USA

Introduction: The frequency of microdeletions in the Azoospermia Factor (AZF) region on the long arm of the Y chromosome ranges between 2 and 20% in subfertile men, depending on clinical selection criteria and the deletion detection protocol. Deletions have been associated with a range of male reproductive pathology (oligozoospermia to complete germ cell aplasia). In cases of hypospermatogenesis, successful treatment depends on intracytoplasmic sperm injection (ICSI); however, this procedure carries the real risk of perpetuating Y-linked mutations in the male progeny. Screening for AZF microdeletions is becoming standard practice prior to ICSI, and this provides information that is critical for accurate genetic counselling. At present, two systems are being considered for genetic analysis of the Y chromosome, namely the 'home brew' system composed of randomly selected Sequence Target Sites (STS) which may or may not accurately diagnose infertility associated microdeletions, and a commercially available system YDDS, composed of 22 STS in five multiplex reactions with positive controls. In this study we report our experience in assessing microdeletions in AZF and establishing Y-optimal deletion detection protocol for use in DNA derived from a range of templates.

Materials and methods: Blood samples were obtained from the male partner of 16 infertile couples (one azoospermic, seven oligozoospermic, eight normozoospermic). Buccal cells were obtained from 12 of these men, and spermatozoa from five. After extraction, DNA samples were screened using both a 'home-brew' multiplex screening system, and YDDS (a commercially available kit; Y Chromosome Deletion Detection System, Version 2.0, Promega, Corp.). Loci tested included those currently being assessed as a standard by the European Academy of Andrology and the European Molecular Genetics Quality Network.

Results: Using the 'home-brew' method, Y-microdeletions were detected in leukocytes of three of the 16 men, whereas the YDDS revealed that this was an accurate diagnosis in only one of the three men, namely an azoospermic patient with a large deletion involving AZFb and AZFc. Interpretation of amplifications from sperm and buccal cells using the 'home-brew' method was impossible due to a high amplification background and PCR failure, including amplifier dro

P-out. In addition, reproducibility across experiments was low. The large deletion was defined as extending from proximal AZFb (sY 121) to distal AZFc (sY 157) was confirmed using YDDS in leukocyte DNA. In samples from the remaining 15 individuals the Y chromosome was found to be normal. Robust amplification was obtained in the case of all 22 loci and in the positive control.

Conclusion: The Y-microdeletion detection rate (6.2%) is within the expected range for a non-selected male population. Standardization of methodology both in terms of accuracy and precision is critical, and could not be achieved using a 'home-brew' method. The false-positive error rate was high when samples were screened using this method. Screening of samples using the YDDS produced reliable results, and accurately portrayed the Y-chromosome using only pathology associated STS distributed in all AZF subregions, including the STS employed in the European standardization effort. Y-microdeletion assessment in the semen sample, and particularly in the single spermatozoon, remains the ideal goal. Successful amplification of buccal cells provides an immediate screening of the father/son couplets to identify mutation transmission.

P-097. High pregnancy risk and poorer perinatal outcome after IVF

Beck A.[1], Herrero J.[1], Stalf T.[2], Mehnert C.[2], Gips H.[2] and Lang U.[1]

[1] Department of Obstetrics and Gynaecology, Justus-Liebig-University Giessen and [2]Institute of Reproductive Medicine, Giessen, Germany

Introduction: Pregnancy after assisted reproduction [IVF/intracytoplasmic sperm injection (ICSI)] are considered to be 'risk' pregnancies. The goal of our study was to assess the evolution of the pregnancy and how the perinatal outcome after IVF might differ from that of pregnancies not conceived through assisted reproduction.

Materials and methods: Between 1994 and 1999, 406 pregnancies after IVF were examined retrospectively. Standard questionnaires were sent to the referring gynaecologist and the patients. Data were compared with those of 338 737 patients without any sterility treatment of the perinatal survey of the state of Hessen/Germany (HEPE) for the same period. Furthermore, the influence of spermiogram parameters on the pregnancy and perinatal outcome was analysed. The cut-off levels were 10×10^6 spermatozoa/ml, 10% motility, and 10% normal morphology.

Results: Some 300 (73.9%) of the pregnancies after IVF were singleton pregnancies, 97 (23.9%) were twins, and nine (2.2%) were triplets. A high rate of bleeding was found at the beginning of the pregnancy (IVF 15.7% versus HEPE 2.5%; $P < 0.05$), hypertension (IVF 6.3% versus HEPE 2.1%; $P < 0.05$), gestational diabetes (IVF 4.0% versus HEPE 0.8%; $P < 0.05$), and premature labour (IVF 25.7% versus HEPE 7.3%; $P < 0.05$) in the IVF group. As a consequence of the high number of risk pregnancies after IVF, a high prematurity (IVF 20.3% versus HEPE 6.7%; $P < 0.05$) and Caesarean section rate (IVF 35.7% versus HEPE 19.9%; $P < 0.05$) was found. The perinatal mortality in the IVF group was 1.0% (HEPE 0.6%). Furthermore, 16.8% of the newborns after IVF were growth-restricted (HEPE 8.0%; $P < 0.05$), and 4.4% were severely growth-restricted (under 3rd percentile). These significant differences for pregnancy variables and perinatal outcome were also observed after matching for parity and age in the analysed groups. Similar results were observed when comparing twin pregnancies after IVF with twin pregnancies of the HEPE group. No significant differences were found in the IVF group with regard to the method of fertilization (IVF/ICSI). Patients with polycystic ovaries (PCO) had higher rates of pregnancy hypertension (10.8 versus 5.9%) and gestational diabetes (9.6 versus 3.4%; $P < 0.05$). Patients who underwent ovarian stimulation had a higher rate of multiple pregnancy (38.7 versus 23.3; $P < 0.05$), and therefore a higher prematurity rate (53.8 versus 38.8%; $P < 0.05$). Pregnancies conceived with spermatozoa where normal morphology was <10% had an even higher rate of hypertension (8.8 versus 5.9%) and intrauterine growth restriction than those with better spermatozoa morphology (25.6 versus 7.7%; $P < 0.05$). Further spermiogram parameters, such as the number and motility of the spermatozoa, had no significant influence on the pregnancies.

Conclusion: Pregnancies after IVF therapy are high-risk pregnancies whether compared to a standard collection (HEPE) after matching number of fetuses, age and parity of the patient. No differences in pregnancy course and outcome were found with regard to the method of fertilization. Patients with PCO showed high rates of hypertension and gestational diabetes, most likely due to their insulin resistance. The high rate of hypertension and intrauterine growth restriction in pregnancies achieved with an ejaculate having sperm of <10% normal morphology might indicate a disturbed placentation.

P-098. Haemostasis parameters during ovarian stimulation for IVF: results of a prospective study

Fritz K.[1], Herrero J.[1], Stalf T.[2], Lang U.[1], Gips H.[2] and Kemkes-Matthes B.[1]

[1]Department of Internal Medicine, Department of Gynaecology, Justus-Liebig-University, Giessen and [2]Institute of Reproductive Medicine, Giessen, Germany

Introduction: Although thromboembolic events have been observed during IVF hyperstimulation, little is known about coagulation changes during IVF. Therefore, an attempt was made to determine the effects of two different stimulation protocols on the coagulation system in women undergoing an IVF programme.

Materials and methods: Plasma concentrations of Quick-value (PT), aPTT, fibrinogen, tissue factor (TF), tissue factor pathway inhibitor (TFPI), prothrombin-fragment F1+2, D-dimer, oestrogen, progesterone, LH, FSH, androgens and prolactin were measured before IVF stimulation, twice during stimulation, and at 3 days after ovulation. Patients with hereditary thrombophilic coagulation defects were excluded. Twenty-five infertile women, aged 22–40 years and undergoing IVF, were included. Twelve women formed a 'short-protocol' group (SPG) and underwent stimulation with human menopausal gonadotrophin (HMG; Menogon®) in combination with Flare-up gonadotrophin-releasing hormone (GnRH) analogue (Suprecur®). Another 13 women formed a 'long-protocol' group (LPG) and underwent stimulation with HMG and down-regulation therapy with leuprorelin (Enantone Gyn®).

Results: No increases in either D-dimer or F1+2 concentrations were observed in both groups during the first hyperoestrogenic phase before ovulation. However, following ovulation a significant increase in D-dimer concentration was observed in the SPG (191.5 – 406.5 ng/ml; $P = 0.001$), and an even greater increase in the LPG (180 – 875 ng/ml; $P < 0.001$). This increase correlates with the increase in progesterone concentrations in the SPG (3.0 – 48.5 ng/ml; $P < 0.001$) and LPG (1.4 – 116.0 ng/ml; $P < 0.001$). Furthermore, a significant increase of F1+2 was observed in both groups [SPG 0.6 – 0.95 nmol/l ($P = 0.05$); LPG 0.5 – 0.7 nmol/l ($P < 0.001$)]. This increase was significantly correlated with progesterone concentrations. Higher concentrations of fibrinogen and progesterone were seen in LPG patients compared with SPG patients ($P < 0.03$). No correlations were found between concentrations of either LH, FSH, androgens or prolactin, and coagulation parameters.

Conclusion: The present study indicates induction of haemostasis activation after ovulation due to ovarian stimulation. The parameters of coagulation activation correlate with increasing progesterone concentrations during IVF treatment.

P-099. Percutaneous epididymal sperm aspiration versus testicular sperm extraction in azoospermic patients

Hammad F.A., Abu-Khizaran A., Hussieni J. and Abu-Khizaran S.

Razan Medical Centre for IVF, Nablus, Palestine

Introduction: Since the introduction of intracytoplasmic sperm injection (ICSI) forthe treatment of infertile couples, many procedures have been introduced to retrieve spermatozoa from 'azoospermic' men. Percutaneous epididymal sperm aspiration (PESA) was pioneered by the London Fertility Centre, and has been used at our centre since 1998. Patients with negative PESA undergo testicular sperm extraction (TESE).

Materials and methods: The records of 293 azoospermic patients covering the period from June 1998 until December 2000 were reviewed retrospectively. In total, 158 patients were categorized as obstructive, and PESA was positive in 120 (75.9%) of these. Some 135 patients were categorized as unobstructive, and PESA was positive in 42 (31.1%) of these. A total of 131 patients from both groups underwent TESE; 19 patients had no spermatozoa and were excluded from the study.

Results: In the PESA group ($n = 162$), 1738 metaphase II oocytes (MII) were injected, 1076 were fertilized (61.9%), 918 embryos were cleaved (85.3%), and 550 embryos were transferred (3.4 per patient). Fifty-six patients became pregnant (pregnancy rate 34.5%), and 83 gestational sacs were seen, with an implantation rate of 15.1%. In the TESE group ($n = 131$), 112 patients had spermatozoa, 1225 (MII) oocytes were injected, 492 were fertilized (40.1%), and 435 embryos were cleaved (88.4%). Subsequently, 240 embryos were transferred (1.8 per patient). Thirteen patients became pregnant (pregnancy rate 11.6%), and 16 gestational sacs were seen, with an implantation rate of 6.7%.

Conclusion: PESA, as we might expect, is more successful in obstructive than unobstructive azoospermic patients (75.9 versus 31.1%). However, PESA should always be attempted first as it is simple and minimally invasive and has a high success rate. In our hands, when ICSI is performed with PESA spermatozoa the fertilization rate is significantly higher than with TESE spermatozoa, and the implantation rate is almost double.

P-100. Factors affecting the success of cryopreservation of human embryos

Salumets A., Tuuri T., Mäkinen S., Husu L., Kotola S., Tainola R., Santti K., Suikkari A-M.

The Infertility Clinic of The Family Federation of Finland, PO Box 849,00101 Helsinki, Finland

Introduction: Cryopreservation of supernumerary human embryos is an essential part of IVF and ICSI. To better select embryos for freezing, the factors affecting the success of cryopreservation need to be investigated. We asked the question which embryos should be frozen? To find an answer the present retrospective study was designed to determine the influence of developmental stage and quality of embryos upon the pregnancy outcome of frozen embryo transfers.

Materials and methods: A total of 1896 cryopreserved IVF/ICSI embryos were thawed during 1998–1999. Cryopreservation of all embryos was carried out using the 1,2-propanediol protocol: described by Lassalle *et al.*. Only grade 1 (no fragments), 2 (<20% fragmentation) and 3I (20–35% fragmentation) cleavage stage embryos were frozen. The embryo survival rate (at least 50% of blastomeres surviving), clinical pregnancy rate and implantation rate were calculated for pronuclear, day two (D2) and day three (D3) cleaved embryos. A total of 378 D2 two-embryo transfers were divided into three groups according to the quality of embryos prior to freezing. In group 1, two good quality (grade 1 and 2) embryos were transferred, in group 2 one good quality and one moderate quality (grade 3I) embryos were transferred, and in group 3 only moderate quality embryos were transferred.

Results:

Table I. Embryo survival, clinical pregnancy and implantation rates for pronuclear, D2 and D3 frozen-thawed embryo transfers

The embryo survival rate (%) **	No. of embryos transferred	Transfers (*n*)	Clinical pregnancies	(%) *	Implantation (%)*
2PN	271/321 (84.4)	229	135	24/135 (17.8)	11.8
D2	1094/1419 (77.1)	920	542	95/542 (17.5)	11.4
D3	89/156 (57.1)	74	47	4/47 (8.5)	6.8

**$P < 0.005$; *no significant differences

TableII.

Group	Embryo survival (%)***	No. of embryos transferred	Transfers (*n*)	Clinical pregnancies (%)	Implantation rate (%)*
1	82.4	262	131	21/131 (16.0)**	9.5
2		270	135	35/135 (25.9)**	14.1
3	73.2	224	112	20/112 (17.9)	10.3

*'**$P = 0.000$; **$P < 0.05$; *no significant differences

Conclusions: Our results show that the survival of cryopreserved embryos depends on their developmental stage. The embryo survival was significantly higher for pronuclear than cleavage stage embryos and likewise it was higher for day two compared with day three embryos. As expected, the good quality (grade 1 and 2) embryos survived better than moderate (grade 3I) quality embryos However, cryopreservation did not compromise the viability of surviving embryos as the clinical, pregnancy and implantation rates were similar regardless of embryo quality or developmental stage at freezing.

P-101. Should ICSI be the treatment of choice in isolated teratozoospermia?

Strassburger D., Raziel A., Friedler S., Schachter M., Bern O., Komarovsky D., Kasterstein E. and Ron-El R.

Infertility and IVF Unit, Assaf Harofeh Medical Center, Tel-Aviv University, Israel

Introduction: Although intracytoplasmic sperm injection (ICSI) is considered first-line therapy for severe male factor, fertilization after conventional IVF is still generally considered preferable. Comparative studies of 'sibling' oocytes undergoing ICSI or IVF demonstrated inconclusive results when teratozoospermia was the only abnormal semen parameter. The aim of our study was to examine the effects of different degrees of isolated teratozoospermia on the outcome of conventional IVF and ICSI using 'sibling' oocytes.

Materials and methods: The study comprised 246 cycles among patients whose major indication for treatment was teratozoospermia. The average normal sperm morphology (Kruger criteria) was 6.6 ± 1.9%. The normal form count was ⩽5% in 73 cycles, 6–7% in 90 cases, and 8–10% in 83 cases. Sperm concentration was $>10\times10^6$ /ml in all cycles (mean, 53.8 ± 40.3×10^6/ml). Motility was> 35% (mean, 54.7 ± 20.7%). The control group consisted of 48 cycles in patients who desired IVF and ICSI treatment on their 'sibling' oocytes with normal sperm parameters. Semen preparation, oocyte retrieval, IVF, and ICSI were performed in the routine mode of our programme. A total of 3807 study group oocytes was divided into 1648 for conventional IVF and 2159 for ICSI. In the control group, 767 oocytes were divided into 356 for IVF and 411 for ICSI. Assessment of fertilization, embryo cleavage, number of cells on day2, and quality (grades 1–4) was performed and compared between IVF and ICSI treatments.

Results: 157 of the 246 (64%) cycles achieved fertilization by conventional IVF, while in the control group 34 of 48 cycles (71%) achieved fertilization $P = 0.384$). All ICSI cycles in both study and control groups had at least one fertilized oocyte (ICSI versus IVF, $P < 0.001$). The

following Table shows the fertilization and the transferred embryo quality in the different groups.

Embryo cleavage varied between 94 and 99% with no statistical significance among the groups. Of a total of 239 transfers, only-IVF embryos were transferred in 26 cycles (11%), only-ICSI embryos in 149 cycles (62%), and ICSI with IVF embryos (mixed group) in 64 cycles (27%). No embryos were available for transfer in 3% of the cycles. One hundred and sixty-one (28%) of 570 IVF embryos were deemed best for transfer, while among the 1390 ICSI embryos, 534 (38%) were deemed best for transfer ($P<0.03$). One hundred and seventy-seven (31%) and 582 (42%) IVF and ICSI embryos respectively, were of freezing quality ($P < 0.0001$).

Normal forms	Fertilization/oocyte (%)		No. cells/embryo*		Embryo quality*	
	IVF (cycles with 2PN)	ICSI	IVF	ICSI	IVF	ICSI
⩽5	150/312 (48)[a]	495/722 (69)[a]	3.6	4.3	1.7	1.7
>5⩾7	313/554 (56)[a]	548/769 (71)[a]	3.9	3.9	1.7	1.7
⩾8⩽10	223/408 (54)[a]	441/668 (66)[a]	3.9	3.9	1.8	1.8
Total	686/1274 (54)[a]	1484/2159 (69)[a]	3.8	4.0	1.7	1.7
Control	132/256 (52)[a]	286/411 (70)[a]	3.9	3.7	1.8	1.8

a, $P < 0.0001$; * mean values.

Conclusions: In cases where teratozoospermia is the only abnormal semen parameter, the fertilization rate in conventional IVF, as well as the number of embryos suitable for transfer and freezing is significantly lower in comparison to ICSI, even when cycles with complete fertilization failure are not considered. However, the severity of the teratozoospermia in itself does not effect the fertilization rate of either conventional IVF or ICSI. Thus the percentage of normal forms cannot be used as a guideline for the mode of treatment, and therefore ICSI should be viewed as the treatment of choice for patients, regardless of sperm quality.

P-102. Efficacy of laser zonal thinning for frozen–thawed human embryos: prospective, controlled, and randomized study

Mauri A.L.[1], Petersen C.[1], Ferreira R.C.[1], Baruffi R.L.R.[1] and Franco J.G. Jr [1,2]

[1] Center for Human Reproduction, Sinhá Junqueira Maternity Foundation, R. D. Alberto Gonçalves 1500, 14085–100; and [2] Department of Obstetrics and Gynecology, University of Ribeirão Preto (UNAERP), Ribeirão Preto, SP, Brazil

Introduction: It has been suggested in the literature that the process of embryo cryopreservation may affect the physicochemical characteristics of the zona pellucida (ZP), causing its hardening, and apparently impairing blastocyst hatching, but the physiology is not reliably documented. It has also been theorized that creating an artificial opening (assisted hatching, AH) in the ZP allows the embryo to hatch after blastocyst formation. Some studies have demonstrated that the use AH for thawed human embryos can improve pregnancy and implantation rates. However, AH has been carried out using various methods whose clinical relevance in a cryopreservation programmes remains controversial and undefined. The object of this study was to evaluate, in a prospective and randomized manner the efficacy of one AH method, laser zonal thinning (L-ZT), applied to thawed embryo transfers (ETs) compared with thawed ETs not submitted to AH.

Materials and methods: A total of 70 patients who were submitted to an ICSI programme and had their excess embryos frozen and thawed by the slow method were divided in a prospective and randomized manner (3:1) into two groups at the time of receiving their thawed embryos: group I, 53 patients whose thawed embryos transferred were all submitted to L-ZT; group II, 17 patients whose thawed embryos transferred were not submitted to L-ZT (control group). In group I, L-ZT was performed using a non-contact laser with length of 1.48 µm (Fertilaser) with 1–2 irradiations of 10 ms applied to the ZP of each embryo to thin 60–80% of the ZP. Embryo transfer was performed soon after the measurement of ZP thickness using an eyepiece with a micrometer scale, and L-ZT procedure. Data were analysed by the Mann–Whitney test.

Results: Clinical and laboratory details of the studied groups are shown in Table 1

	Group I (L-ZT)	Group II (Not L-ZT)	*P*
Patients	53	17	
Age	32.9 ± 5.0	34 ± 3.2	NS
Embryo survival (%)	93	96	NS
Embryo cleavage (%)	68	61 NS	
ZP (µm)	17.8 ± 3.1	17.3 ± 2.5	NS
Cleavage embryos transferred	1.9 ± 0.9	2.5 ± 1.0	NS
Implantation rate (%)	9.6	10.5	NS
Pregnancy rate (%)	17	17.6	NS

Conclusion: Pregnancy and implantation rates were not different between the group submitted to L-ZT and the control group. In the present study, the use of L-ZT was not effective for frozen–thawed embryos.

P-103. The adjunctive use of metformin in PCOS patients undergoing ICSI treatment: preliminary data in 47 cycles

Kilani Z., Shaban M., Dakkak A., Al-Khatib I., Abu Samen A., Al-Rifai M. and Haj Hassan L.

The Farah Hospital, Zahran, P.O. Box 5323, Amman 11183 Jordan

Introduction: Polycystic ovary syndrome (PCOS) is a common endocrinopathy affecting women of reproductive age. It is characterized by hyperandrogenism, chronic anovulation, and infertility. Insulin resistance is common in such patients (70%). The use of insulin sensitizing agent (metformin) in PCOS reverses the endocrinopathy, restores normal menses, and improves fertility and delivery rates. Data regarding the use of such agents in ICSI cycles is quite limited.

Materials and methods: Forty-seven PCO patients with insulin resistance were started on oral metformin 500 mg three times daily, 1–3 months prior to their ICSI cycles. The average age of patients was 29.4 ± 5.6 years and the average weight was 85.7 ± 19.6 kg. Ovulation induction was carried out using either the long or the short protocols. Metformin therapy was continued all through the induction period until 12 days after embryo transfer.

Results: The adjunctive use of metformin was assessed in 47 ICSI cycles in PCO patients with insulin resistance. A detailed analysis of the results is shown in the Table.

Total cycles	47
Average age (years)	29.4 ± 5.6
Average weight (kg)	85.7 ± 19.6
HMG duration (days)	11.8 ± 6.1
MII oocytes (average)	359 (7.3 ± 4.4)
G1 & G2 oocytes	56.9%
Fertilization rate	69.4%
Total embryos	243
Type I & II embryos (%) 159 (65.4%)	
Transferred embryos (average)	120 (2.6 ± 0.8)
Implanted embryos (%)	29 (24.2%)
Total pregnancies	28
Biochemical	6
Abortion	4
Ongoing	17
Delivered	1 (twins)

Conclusion: Preliminary data regarding the adjunctive use of metformin in PCO patients with insulin resistance in ICSI cycles is associated with a good-quality oocytes and good fertilization rates and embryo development. It is associated with high pregnancy rate. More data is needed to evaluate metformin in controlled trials.

P-104. Cessation of gonadotrophin-releasing hormone (GnRH) agonist therapy combined with high-dose urinary gonadotrophins does not improve the outcome of IVF–ET cycle in poor responders

Karacan M., Demircan A., Karabulut O., Kervancıoğlu E. and Benhabib M.

Assisted Reproduction Unit, International Hospital, Yesilyurt, Istanbul, Turkey

Introduction: Different controlled ovarian hyperstimulation (COH) protocols have been examined to increase success rate in poor responders. It

was postulated that cessation of gonadotrophin-releasing hormone (GnRH) agonist following mid-luteal suppression of endogenous gonadotrophins may enhance ovarian response.

Materials and methods: In our study, 44 women who had previously undergone COH with standard luteal phase long protocol and were either cancelled or had had less than three oocytes in the previous cycle were included. Within 3 months after the failure, all women were scheduled for the new protocol. GnRH agonist (Lucrin, s.c., 0.5 mg daily) was started on cycle day 21 and continued until the first day of menstrual bleeding. High-dose urinary gonadotrophins (8 ampoules/day) were administered beginning on day 3 and the cycle was monitored by vaginal ultrasound and oestradiol assessments. Cycles with less than three follicles and oestradiol concentrations of less than 200 pg/ml on day 7 were cancelled.

Results: Average age and day 3 FSH level of the women were 37.5 ± 3.7 and 9.4 ± 2.0 respectively. Average duration of GnRH agonist use was 10.8 ± 2.0. During COH 16 (36.3%) cycles were cancelled and 28 women underwent oocyte retrieval (average number of follicles obtained and oocytes collected were 4.2 ± 2.6 and 3.9 ± 2.2 respectively), in four women no oocytes were obtained. Fertilization failure occurred in three women and in 21 women embryo transfer was performed with the average number of embryos transferred 2.3 ± 1.2. Two pregnancies were achieved, one of them aborted. Overall, one single baby was obtained in 44 women, and the take-home baby rate per cycle started was 2.2%.

Conclusions: Cessation of GnRH agonist following luteal-phase down-regulation does not yield favourable pregnancy rates in women who displayed poor response in previous long protocol and COH cycles.

P-105. Low-dose luteal gonadotrophin-releasing hormone (GnRH) agonist in assisted reproductive technology (ART)

Depalo R., Loverro G., Giannelli L., Lorusso F., Mei L. and Selvaggi L.

Department of Obstetrics and Gynaecology, University of Bari, Italy

Introduction: The use of GnRH agonist in ART protocols for ovarian stimulation with gonadotrophins is associated with an increase in follicular recruitment and prevention of the spontaneous LH. The long luteal protocol is generally the most effective and the most commonly used at present but it has the disadvantage of a relatively high cost due to the increased requirement of gonadotrophins. Moreover, the GnRH agonist doses generally used in ART may be excessive and result in pituitary over-suppression. Aim of this study was to assess whether lower doses of GnRH agonist may be sufficient to prevent the LH peak without inducing pituitary over-suppression, while still ensuring adequate oocyte retrieval.

Materials and methods: We performed a prospective study on 73 women undergoing IVF. Polycystic ovarian disease and syndrome patients, poor responders, patients with endometriosis and patients with a body mass index (BMI) >28 were excluded. Forty-five patients received a subcutaneous tryptorelin depot (Decapeptyl 3.75; Ipsen SpA) and 28 patients subcutaneous tryptorelin acetate daily (Decapeptyl 0.1 mg) starting from day 21 of the previous cycle. From day 2 of the new cycle, if the blood concentrations of oestradiol were <30 pg/ml, patients received two or three ampoules of gonadotrophin administered daily for 5 days, after which the dose was individualized according to the ovarian response. In the group of patients taking tryptorelin daily, the dose was decreased to 0.5 mg/day and then stopped on the day of administration of HCG.

Results: No differences between the groups were detected with respect to diagnosis, patient's age, or pregnancy rate. In the low-dose group, the mean number of total gonadotrophin ampoules and of days of stimulation was significantly less, and the oestradiol peak and number of oocytes retrieved, fertilized, and cleaved were significantly greater.

Variable	Depot GnRHa	Low dose GnRHa	*P* value
Age	32	31	NS
Total stimulation 4.3 (ampoules)	3.2	0.032	
Total stimulation 12.3 (days)	11	0.067 (n.s.)	
Oestradiol peak	1518	2207	0.003
No. oocytes retrieved	7	9.4	0.039
No. oocytes fertilized	3.3	6	0.0024
No. oocytes cleaved	2.6	5.3	0.0011
Pregnancy rate (%)	28	26	NS

GnRHa = GnRH agonist

Conclusion: Pituitary over-suppression induced by GnRH agonist causes an increase in the gonadotrophin requirement for ovarian stimulation and a reduction in the number of oocytes retrieved and fertilized. Pituitary over-suppression could occur after excessive or prolonged doses of GnRH agonist administered according to the long luteal protocol. There is an increased risk of over-suppression in normal-weight or under-weight women, as their smaller adipose mass means that there is greater bioavailability of the peptide and hence an increase in the circulating levels of GnRH agonist, which could significantly affect extra-pituitary GnRH receptors. In conclusion, the best response to ovarian stimulation in ART cycles depends on many factors, not least of which is the identification of the best GnRH agonist dose.

P-106. Addition of HCG to progesterone as a luteal support improves pregnancy rates for patients with low mid-luteal oestradiol levels in IVF and ICSI

Fujimoto A., Osuga Y., Ooi N., Fujiwara T., Yano T. and Taketani Y.

Department of Obstetrics and Gynecology, University of Tokyo, Tokyo, Japan

Introduction: The effects of several luteal support protocols for IVF and ICSI are controversial. Progesterone in the luteal phase has been considered as essential for pregnancy and oestradiol only as permissive. The aim of this study was to assess the relationship between mid-luteal oestradiol levels and pregnancy rates in IVF and ICSI cycles supported with only progesterone, and to investigate the effect of the addition of HCG to progesterone as a luteal support for patients with low mid-luteal oestradiol levels in previous cycles.

Materials and methods: Clinical pregnancy rates of 436 cycles supported with only progesterone (25 mg i.m. daily) were evaluated with oestradiol levels on day 7 after embryo transfer (ET). Patients with previous failed cycles with low mid-luteal oestradiol levels (<100 pg/ml) were randomly allotted to two different luteal support protocols: progesterone only (P protocol), and additional HCG (3000 IU i.m. on days 1, 4 and 7 after embryo transfer) on progesterone (P+HCG protocol) in the next IVF or ICSI cycle. Clinical pregnancy rates were compared using chi square test.

Results: As shown in Table 1, pregnancy rate was significantly low in patients with low mid luteal oestradiol levels (<100 pg/ml) compared to other groups with higher oestradiol levels. In patients with low mid-luteal oestradiol levels in the previous cycles, P+HCG protocol showed higher pregnancy rates than P protocol (Table 2).

Table 1

Oestradiol on day 7 after ET (pg/ml)	0–25	26–50	51–100	101–200	201–500	501–1000	⩾ 1001
No. of transfer cycles	65	70	83	70	57	44	47
Clinical pregnancy	5	7	17	19	15	17	16
Clinical pregnancy/ET (%)	7.7	10	20.5	27.1	26.3	38.6	34

ET = embryo transfer

Table 2.

	Progesterone	P+HCG
No. of transfer cycles	51	73
Clinical pregnancy	7	21
Clinical pregnancy/ET (%)	13.7	28.8

$P < 0.05$

Conclusion: In controlled ovarian stimulation protocol, not only progesterone but also oestradiol may play an important t role for pregnancy. For patients with low mid-luteal oestradiol levels in progesterone-only supported cycles, addition of HCG to progesterone appears to improve pregnancy rates.

P-107. Friendly IVF: patient opinions. Results of a questionnaire study comparing attitudes among patients treated with IVF in the natural cycle or following clomiphene citrate stimulation compared with long down-regulation and FSH

Højgaard A.D.[1], Ingerslev H.J.[1] and Dinesen J.[2]

[1]Fertility Clinic, Aarhus University Hospital, Skejby Sygehus, DK-8200 Aarhus N; [2]Perinatal Epidemiological Research Unit, Aarhus University Hospital, Aarhus, Denmark

Introduction: Friendly IVF protocols yield a lower clinical pregnancy rate per started cycle due to a higher cancellation rate. The aim of the study was to point out problem themes and patient preferences in a low-stimulation (LS) regimen compared to a standard (S-IVF) regimen, concerning hormone side-effects, necessity of more treatment cycles, stress related to treatment and risk of cycle cancellations.

Materials and methods: An anonymous 23-item questionnaire was sent to 167 patients randomized to either clomiphene citrate (100 mg on cycle days 3–7) or to natural cycle IVF (LS group) and a historical control group consisting of 116 patients having their first IVF treatment (S-IVF group), both fulfilling the same criteria regarding age (<35 years.) and diagnosis (unexplained, tubal factor or indication for ICSI). The questions related to the latest treatment cycle and preferences of treatment. Scores were measured in a 5-point Likert-type scale.

Results: The response rate was significantly higher in the LS-group (141/167 = 84%) compared to the S-IVF group (66/116 = 57%). Approximately two-thirds of all responders in both groups deemed hormone side effects important but the proportion of patients having unacceptable/severe hormone side effects were significantly smaller in the LS versus the S-IVF group 4/75 (5%) versus 38/63 (60%). Only in the S-IVF group was the reported severity of side effects positively correlated to treatment more than once. Stress due to cycle cancellation was acceptable/not perceptible in 36/75 (48%) of LS responders but only in 8/31 (26%) of S-IVF responders. LS patients had a marked tendency to accept a higher number of treatments than S-IVF patients. A larger proportion in both LS and S-IVF responders would prefer a combination of LS and S-IVF as future treatment options (56% versus 48%).

Conclusion: patients receiving an LS protocol accept repeated cycles. LS should become an integrated part of infertility treatments for those couples that consider avoidance of hormone side effects as more important than high treatment efficacy and low risk of cancellation.

P-108. Sharing oocytes from a single donor: predictors of discordant outcome

Requena A.[1], Isaza V.[1], Caligara X.[2], García-Velasco J.A.[1], Martinez-Salazar J.[1], Landazabal A.[1], Remohí J[2,3], and Simón C[2,3]

[1]IVI-Madrid, Madrid, [2]Instituto Valenciano de Infertilidad (IVI), and [3]Department of Paediatrics, Obstetrics and Gynaecology, Valencia University School of Medicine, Valencia, Spain

Objective: To compare specific characteristics of paired recipients sharing oocytes obtained from a single donor in order to elucidate the variables that influence the outcome in this unique homogeneous population.

Materials and methods: Two hundred and ninety-three paired recipients who shared oocytes from a single donor from September 1999 to December 2000 were retrospectively studied. Discordant outcome was established when at least one of the recipients achieved pregnancy while the other(s) did not. Patients were divided into two groups: group 1 (153 patients) who had positive pregnancy outcome, and group 2 (140 patients), who had a negative pregnancy outcome. Variables evaluated included: age of the recipient, endometrial thickness and ultrasonographic pattern, serum oestradiol levels, presence or absence of male associated factor, number of oocytes received, fertilization rate, number of embryos transferred, type of catheter used at embryo transfer, and day of the transfer. Hormonal replacement therapy was administered as previously described, micronized progesterone was started the day of oocyte donation, and embryo transfer was performed according to each patient's individual programme on day 2, 3, 5, or 6. The paired *t*-test was used and a *P* value < 0.05 was considered significant.

Results: See table

	Group 1 (n = 153)	Group 2 (n = 140)	*P*
Age 37.5 ± 0.4	36.9 ± 0.4	NS	
Endometrial thickness (mm)	9.4 ± 0.2	9.4 ± 0.2	NS
Serum oestradiol (pg/ml)	345.3 ± 28	365.1 ± 32.2	NS
Days on waiting list	37.2 ± 1.5	37.4 ± 1.5	NS
Male associated factor	68/153 (44.4%)	58/140 (41.4%)	NS
Oocytes received (n)	7.6 ± 0.1	7.8 ± 0.1	NS
Fertilization rate	87.3 ± 2.3	79.7 ± 3.2	NS
Embryos transferred (n)	3.4 ± 0.04	3.3 ± 0.04	NS
Day 2 embryo transfer	112/153 (73.2%)	91/140 (65%)	NS
Day 3 embryo transfer	28/153 (18.3%)	40/140 (28.5%)	NS
Day 5 embryo transfer	2/153 (1.3%)	1/140 (0.7%)	NS
Day 6 embryo transfer	11/153 (7.2%)	8/140 (5.8%)	NS
Embryo transfer			
Soft catheter	141/153 (92.1%)	130/140 (92.8%)	NS
Rigid catheter	12/153 (7.9%)	10/140 (7.2%)	NS

Conclusions: When sharing oocytes from a single donor, no independent variable could predict the discordant outcome observed in the recipients. The outcome of paired recipients is not affected by the pregnancy result of each other.

P-109. An open-label multicentre, randomized, parallel, controlled phase II study to assess the feasibility of a new programming regimen using an oral contraceptive prior to the administration of recombinant FSH and a GnRH-antagonist in patients undergoing ART (IVF-ICSI) treatment

van Loenen A.C.D.[1], Huirne J.A.F.[1], Schats R.[1], Donnez J.[2] and Lambalk C.B.[1]

[1]Research Institute for Endocrinology, Reproduction and Metabolism, VU Medical Centre, Department of Obstetrics and Gynaecology, Division of Reproductive Medicine, PO Box 7057, 1007 MB Amsterdam, The Netherlands; [2]Universite Catholique de Louvain, Cliniques Universitaires Saint-Luc, Avenue Hippocrate 10, 1200 Bruxelles, Belgium

Introduction: Taking advantage of the endogenous FSH that starts to rise as soon as an oral contraceptive (OC) is discontinued, we began with the administration of rFSH (Gonal-F) immediately after the last administration of OC. We compared the efficacy and convenience of this new programming regimen against a control group. The GnRH-antagonist Antide was used for postponing the spontaneous mid-cycle LH surge during ovarian stimulation. We document further the Antide's safety and tolerability in this indication.

Methods: Sixty-four patients, justifying ART (IVF, ICSI) treatment, were randomized equally to receive either the oral contraceptive programme followed by Gonal-F stimulation or commence Gonal-F immediately without pre-programming of the cycle (Control group). The Gonal-F was administered daily either from day 1–2 after the last OC administration or from day 2–3 of a spontaneous menstrual bleed for patients in the control group. Gonal-F was continued until follicular development was judged to be adequate, as assessed by ovarian ultrasound and serum

oestradiol measurements. Recombinant HCG (Ovidrel) was injected on the day after the last Gonal-F injection, when all of the induction criteria were met. Antide was administered daily from day 6 of the stimulation phase up to and including the day of Ovidrel injection.
Oocytes were retrieved, assessed and fertilized *in vitro* and embryos replaced. Luteal support was given by daily vaginal natural progesterone. Safety and efficacy data were obtained through clinical follow-up, monitoring of adverse events, blood sampling for analysis of LH oestradiol, and progesterone serum levels and ovarian ultrasound.

Results: Following stimulation, the oestradiol levels increased at a slower rate in the OC pre-treated group compared to the control group. In the OC treated group, the duration of Gonal-F treatment was longer, the mean number of follicles $\geqslant$11 mm on day HCG was higher and the mean number of oocytes retrieved was higher compared to the control group.
Interpretation: Oral contraceptive pre-treatment prevents development of potentially dominant follicles that would have determined early timing of HCG administration. Subsequently, over a longer period of stimulation with FSH, more initially smaller follicles are allowed to develop uniformly. This leads to more oocytes at the time of ovum retrieval.

P-110. Inhibin B is a predictive factor for implantation in patients treated with HMG and concomitant Cetrotide® medication for ART

Engel J.B., Smitz J., Felberbaum R., Ludwig M., Reissmann T., Ortmann O., Kohse R. and Diedrich K.

Introduction: Inhibins, heterodimeric glycoproteins, are synthesized by the granulosa cells of the ovary and selectively suppress the pituitary FSH secretion. In the natural cycle, inhibin B is high in the mid-follicular phase and inhibin A reaches its peak in the mid-luteal phase. In ART cycles, inhibin A is correlated with the number of great mature follicles, while inhibin B seems to be associated with the number of smaller follicles. There is still doubt whether inhibins, basal inhibin B in particular, reflect the ovarian reserve. However, in controlled ovarian hyperstimulation (COH) according to the 'long' agonist protocol, it has been shown that throughout the stimulation inhibin B correlates with the number of follicles and the implantation rate. To the best of our knowledge this has not been shown so far in ART using the multiple-dose antagonist protocol.

Methods: We assessed the serum samples of 24 patients. The patients received a COH with HMG and mid-cycle administration of the LHRH-antagonist Cetrotide® according to the multiple-dose protocol. We measured inhibin B at stimulation day 1 and 5, day of HCG administration, day of follicular puncture, day of the embryo transfer, and 7 and 14 days after the transfer using a solid-phase sandwich enzyme-linked immunosorbent assay (Serotec, Oxford, UK).

Results: The mean patients age was 31.4 $\pm$ 3.7. There were 14 cases of male infertility, seven cases of tubal, and three cases of combined infertility. The mean number of ampoules of HMG used per cycle for stimulation was 30.9. Then mean number of follicles $>$18 mm was of 3.9 $\pm$ 1.8 and the mean number of follicles 15–18 mm 3.7 $\pm$ 2.6 respectively. Two biochemical and six clinical pregnancies were achieved. On the day of HCG administration, the serum samples of 18 patients were available. The mean concentration of inhibin B in non-pregnant patients at that day was of 1250 pg/ml. The inhibin B serum concentrations of seven of 10 non-pregnant patients were below that mean, while inhibin B serum concentrations at the day of HCG administration in all clinically or biochemically pregnant patients was at or above that mean.

Discussion: This is the first report about a possible correlation between inhibin B serum concentrations and implantation rates after COH according to the multiple-dose antagonist protocol. Our findings seem to be strong evidence that inhibin B at the day of HCG administration is a positive predictive factor for implantation of the embryo in ART cycles according to the multiple-dose antagonist protocol using Cetrotide® and HMG. Our results suggest that inhibin B could be of clinical importance in ART in the near future. Whether there is a clear cut-off value remains to be assessed in a larger trial.

P-111. Luteal phase support with progesterone in IVF/ET cycles: a prospective, randomized study comparing vaginal and intramuscular administration

Geusa S., Causio F., Marinaccio M., Stanziano A. and Sarcina E.

First Department of Gynecology and Obstetrics, University of Bari, Bari, Italy

Introduction: A large number of studies have demonstrated that a luteal-phase deficiency is a possible problem in women who undergo ovarian stimulation with gonadotrophins. Because of this, a luteal-phase support (LPS) is considered as a standard protocol therapy after ovarian stimulation procedures and during early pregnancy following IVF/ET. Progesterone is considered the drug of choice and it is routinely administered by intramuscular (i.m.) injection. This prospective, randomized study was carried out to assess the efficacy of luteal and early pregnancy support using a progesterone gel preparation designed for vaginal application, as compared with the standard i.m. formulation.

Materials and methods: From June 1999 to June 2000, we evaluated 318 consecutive women undergoing IVF treatment. All patients affected with systemic or endocrine pathologies were excluded. We also excluded women over the age of 42 years. All patients underwent IVF after down-regulation with a GnRH agonist depot formulation and stimulation with recombinant FSH. All 318 patients were randomized to receive once a day progesterone supplementation either by vaginal application at a dose of 90 mg/day or by i.m. injection at a dose of 50 mg/day). Planned treatment was started after oocyte retrieval. The difference between the two groups was analysed by Chi-square test and $P < 0.05$ was considered statistically significant.

Results: Eighteen patients who did not receive an embryo transfer could not be assessed. The two groups of patients were comparable for characteristics (mean age, previous pregnancies and deliveries, and prevalence of primary infertility). The mean number of embryos transferred for each route of progesterone administration was not statistically different (i.m. 3.5 and vaginal 3.2 embryos). The clinical pregnancy rate was similar in both arms of the study, being 28.0% in patients treated with i.m. progesterone (42/150) and 26.7% in patients treated with vaginal progesterone (40/150). For women of all ages, there was no statistical difference in implantation rates for the various routes of progesterone (i.m. 20.2% and vaginal 17.3%).

Conclusion: Our data showed that in women undergoing IVF/ET, implantation and pregnancy rates are similar with the use of i.m. progesterone compared to vaginal progesterone. The former is sometime associated with local pain, the latter is much more expensive. Therefore this study gives additional reasons for post-aspirational support with i.m. progesterone.

P-112. Therapeutic efficacy of TEST-Yolk-treated glass-wool column filtered spermatozoa in IVF

Summers R.[1], Nani J.M.[1], Brasch J.[1], Salhia A.[2], Springer R.S.[1], Jeyendran R.S.[2]

[1]*Advanced Reproductive Health Center, Ltd., Orland Park, IL, and* [2]*Andrology Laboratory Services, Inc., Chicago, IL, USA*

Introduction: The beneficial individual effects of TEST-Yolk treatment and glass wool column filtration (GWCF) procedures on sperm survivability and fertilizing potential is well established. The combination of sperm TEST-Yolk treatment and GWCF prior to sperm penetration assay and human zona binding assay (the combined method) has been demonstrated to significantly enhance assay outcome. However, no data exist from clinical exploration of such TEST-Yolk-treated GWCF spermatozoa on IVF outcome. Such a combined method for sperm processing prior to IVF might improve overall results.

Materials and methods: Twenty-five different ejaculates were divided into two equal halves. One half was then washed and mixed with equal volume TEST-Yolk (Irvine Scientific, Santa Ana, CA). Mixture was then gradually cooled to 5°C in a water jacket and incubated for 1–2 h. An equal volume of IVF media at 37°C was immediately added to warm the

spermatozoa rapidly, followed by washing of the warm mixture. The sperm pellet was then re-suspended in 0.5 ml IVF media, and GWCF (Cook Sperm Filter, Cook Ob/Gyn, Spenser, IN) was then performed. The remaining ejaculate was processed solely through Isolate Density Gradient Centrifugation (Irvine Scientific, Santa Ana, CA). Sperm samples processed by the combined method and the control respectively were utilized in an IVF procedure. Results were then compared to determine relative efficacy.

Results: Spermatozoa separated solely by the isolate procedure resulted in fertilization of 117/137 oocytes (85.4%). Spermatozoa separated by the combined method resulted in fertilization of 122/129 oocytes (94.6%) ($P < 0.5$). Though not statistically significant, a trend toward superior embryo quality (as measured by the number of 8-cell embryos on day 3) was demonstrated by the combined method (52.9%), as compared to embryo quality for spermatozoa separated solely by the isolate procedure (39.7%) ($P = 0.056$).

Conclusions: Spermatozoa processing with the combined method for IVF resulted in a significantly greater fertilization rate than sperm processed solely through the isolate density gradient centrifugation procedure. A trend toward superior embryo quality is observed with the combined method spermatozoa. Overall, this new data suggests a positive therapeutic influence on sperm function for the TEST-Yolk-treated and GWCF separated combined method.

P-113. Hepatitis B and C screening of couples seeking assisted conception. A UK national survey

Marcus S.F.[1], Marcus N.K.[2] and Brinsden P.R.[1]

[1]Bourn Hall Clinic, Bourn, [2]The Surgery, 281 Mill Road, Cambridge, UK

Introduction: The welfare of any child born as a result of assisted reproductive techniques (ART) should be considered prior to starting treatment. The importance of testing couples for hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV) is to enable the couples to make informed decisions as to whether they wish to proceed with their infertility treatment and to inform them of the treatment options available. Furthermore, there are potential risks to staff involved in the cases with positive HBV, HCV, and HIV, and risk of cross-contamination to other gametes and embryos. The objective of this study was to review and analyse the policies of UK licensed IVF centres toward routine screening of infertile couples for HBV and HCV before starting IVF treatment.

Materials and methods: In August 1999, questionnaires were sent to the Medical Directors of all 74 licensed IVF clinics in the UK. The questions were (1) do they routinely screen both partners for HBV and HCV prior to IVF treatment? (2) How do they rate the importance of such screening? (3) Do they have a management policy in place should a test prove positive? (4) How rigidly do they adhere to their protocols? (5) If they do not have a current policy of routine screening, what then are their views about implementing such a policy? (6) What is the size of their clinic as defined by the Human Fertilisation and Embryology Authority (HFEA) in the UK (small centres <200 and large clinics $\geqslant 200$ treatment cycles per year), and (7) Any other comments.

Results: Forty-five of the 74 centres responded to the questionnaires (60.8%). Clinics were classified from A to E according to their screening policy and the type of screening they offer.

Clinic	Number	HBV	HCV	HIV
A	14	Yes	Yes	Yes
B	5	Yes	No	Yes
C	2	Yes	Yes	No
C	2	Yes	No	No
E	22	No	No	No
Total (%)	45	23/45 (51.1)	16/45 (35.0)	19/45 (42.2)

Eighteen clinics rated the screening as essential (40%), eight (18%) as desirable, 10 (22%) as not required, while 9 (20%) did not know. All but two clinics have management protocols if the tests are positive. Two clinics have no current management protocols in place for positive HCV. All clinics but two adhere rigidly to the protocol. These two clinics used the protocols for guidance only. There was no significant difference in the proportion of clinics that routinely carried out screening with regard to the size of their units (46% of small clinics versus 41% of large clinics). The main reasons for not routinely screening infertile couples were: not cost effective, low prevalence of the infectious disease in their population, the requirement and cost of counselling, uncertainty about the need for screening, and potential delay in starting the treatment.

Conclusions: We believe that all patients seeking ART should be given adequate information about HBV, HCV and HIV, the risk of transmission, and testing offered. Not offering the screening deprives infertile couples of the chance of making informed decisions about treatment and denies them the opportunity of taking appropriate measures that could lessen the risk of transmission and slow the progression of the disease.

P-114. Influence of bacterial vaginosis on IVF outcome

Capelli P.[1], Durussel C.[2], Surico N.[3], Bille J.[2], Wirthner D.[1], De Grandi P. [1] and Germond M.[1]

[1]Reproductive Medicine Unit, Department of Obstetrics–Gynaecology, [2]Microbiology Institute, CHUV CH-1011 Lausanne, Switzerland; [3]Clinics of Obstetrics and Gynaecology, Department of Medical Sciences, University of Eastern Piedmont A. Avogadro, Novara, Italy

Introduction: Bacterial vaginosis (BV) develops when the normal vaginal flora of lactobacilli is replaced by an overgrowth of *Gardnerella vaginalis,* Bacteroides, Mobiluncus, *Mycoplasma hominis,* etc. A recent study showed a link between BV and increased abortion rates in IVF. The aim of this study was to evaluate the influence of bacterial vaginosis on the IVF outcome and to provide guidelines for BV screening and treatment prior to IVF in order to improve pregnancy outcome.

Materials and methods: Two hundred and one patients undergoing fresh IVF cycles (121, group A) or in frozen–thawed embryo transfer cycle (80, group B), were recruited between March and December 2000. Before oocyte retrieval (OPU, group A) or embryo transfer (ET, group B) a sample of vaginal discharge was taken with a sterile cotton swab, then smeared on to two glass slides, air dried, and Gram stained. Each different copy of the same slide, read by two observers blind to each other's results, was evaluated for the following morphotypes under oil immersion: lactobacillus, *Gardnerella vaginalis*, Bacteroides, Mobiluncus, and Gram-positive cocci. Each morphotype was quantitated from 1 to 4+ with regard to the number of morphotypes per oil immersion field (0, no morphotypes; 1+, less than 1 morphotype; 2+, 1–4 morphotypes; 3+, 5–30 morphotypes; 4+, $\geqslant 30$ or more morphotypes). A scoring system (0–10) was used to diagnose BV: a score of 0–3 corresponded to a normal vaginal flora, 4–6 to an intermediate stage and $\geqslant 7$ to BV. When the observers disagreed, the slide was reviewed until consensus was reached. Pregnancy was evaluated 14 days after embryo transfer by blood assay. Conception was confirmed by concentration of human chorionic gonadotrophin $\geqslant 10$ IU/l.

Results: The prevalence of normal flora, intermediate stage, and BV in 201 patients was 140/201 (69.6%), 44/201 (21.9%), and 17/201 (8.5%) respectively. Twenty-two slides were read twice before a consensus was reached. Fifty-seven of 201 (28.4%) patients conceived: 43/140 (30.7%) with normal vaginal flora, 10/44 (22.7%) with intermediate vaginal flora, and 4/17 (23.5%) with BV. No difference in conception rate was found between patients with BV and those with normal vaginal flora (relative risk 0.76; odds ratio 0.69). Of the 57 who conceived, 16 (28%) miscarried during the first trimester of pregnancy, 11/43 (25.6%) in the group of normal vaginal flora, 3/10 (30%) in that with an intermediate score, and 2/4 (50%) in BV patients. Women with BV had a risk of miscarriage higher than those with a normal vaginal flora (relative risk 2.15; odds ratio 3.3).

Conclusion: BV does not influence the conception rate of patients undergoing IVF treatment, but it increases the risk of miscarriage in the first trimester of pregnancy. The findings suggest that routine screening for BV is justified and could be implemented during the 6 days before OPU or embryo transfer in order to allow an adequate treatment of BV.

P-115. Prolonged stimulation in assisted reproductive technology: should the cycle be abandoned?

Yulzari-Roll V., Yoeli R., Perri T., Orvieto R., Kerner R., Ferber A., Ashkenazi J., Dekel A., Ben-Rafael Z. and Bar-Hava I.

Department of Obstetrics and Gynecology, Rabin Medical Center (Golda Campus), Petah-Tikva, and Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

Introduction: It is generally accepted that the likelihood for pregnancy is significantly lower when a very long stimulation is needed to achieve a reasonable ovarian response. Therefore, in many assisted reproductive technology programmes, cycles are cancelled when the stimulation period is prolonged. The purpose of this study was to evaluate whether a prolonged cycle should be discontinued because of a low probability of achieving a pregnancy.

Materials and methods: A total of 1787 IVF cycles performed from 1997 to 1999 were studied. The data were collected prospectively on computerized database and evaluated at the end of the study. The patients were divided into two groups according to the treatment received (long or short protocols). Prolonged stimulation was defined as a stimulation period of more than 2 standard deviations of the mean.

Results: No differences in pregnancy rates were detected between women who needed excessive stimulation period and those who did not, both in the short- and long-protocol groups. This was true despite the significantly fewer oocytes retrieved in the women treated with long protocols who needed prolonged stimulation (see Table).

	Long protocol			Short protocol		
	Study	Control	*P* (*t*-test)	Study	Control	*P* (*t*-test)
Patients (*n*)	31	739		36	981	
Total stimulation (days)	16.9	9.8	< 0.0001	15.3	8.5	< 0.0001
Age (years)	34.3 ± 4.6	32.9 ± 5.4	NS	35.3 ± 5.4	35.1 ± 5.7	NS
Oocyte retrieved	7.1 ± 5.2	11.6 ± 6.7	< 0.001	6.2 ± 4.8	7.7 ± 6.0	NS
Fertilization rate (%)	52	50	NS	53	51	NS
Embryos transferred (*n*)	1.9 ± 1.5	2.2 ± 1.7	NS	1.9 ± 1.8	2.1 ± 1.7	NS
Pregnancy rate (%)	25	29.6	NS*	10.8	18	NS*

Figures are means ± standard deviation; NS, not significant; *Chi-square test.

Conclusion: The likelihood of achieving pregnancy was not influenced by the length of stimulation in either the long or the short protocol groups. We therefore recommend that prolonged stimulation IVF cycles should not be discontinued on these grounds alone.

P-116. Does 'coasting' affect the outcome in oocyte donation?

Isaza V.[1], García-Velasco J.A.[1], Scheffer B.[1], Landazabal A.[1], Martinez-Salazar J.[1], Requena A.[1], Remohí J.[2,3] and Simón C.[2,3]

[1]IVI-Madrid, Madrid, [2]Instituto Valenciano de Infertilidad (IVI), and [3]Department of Paediatrics, Obstetrics and Gynaecology, Valencia University School of Medicine, Valencia, Spain

Objective: In order to prevent severe ovarian hyperstimulation syndrome (OHSS), coasting has proved as a convenient way to manage the ovarian hyper-response, avoiding cycle cancellation and elective cryopreservation of all the embryos. We sought to evaluate oocyte quality after coasting in our oocyte donation programme in an attempt to establish if it affects the outcome, while preventing severe OHSS.

Materials and methods: Twelve oocyte donors undergoing controlled ovarian hyperstimulation (COH) with high response (serum oestradiol (E2) >4500 pg/ml) were coasted from August to December 2000. Follicle stimulating hormone (FSH)/human menopausal gonadotrophin (HMG) administration was discontinued while gonadotrophin-releasing hormone analogue was maintained. HCG (10 000 IU i.m.) was administered when oestradiol levels decreased below 3500 pg/ml and oocyte retrieval was scheduled 36 h later. Daily evaluation of oestradiol serum levels was performed. Thirty oocyte recipients shared oocytes from these donors. They received hormonal replacement therapy as previously described.

Results: Donors who underwent coasting were young (25.3 ± 1.4 years), with small body mass indices (21.2 ± 0.8). Polycystic ovarian syndrome subjects were excluded, although 33.3% of the donors exhibited multiple early antral follicles in their basal ultrasound. Coasting outcome was as follows:

After oocyte retrieval, 50% (6/12) of the donors were asymptomatic, 33.3% (4/12) had mild discomfort that was resolved with oral analgesics, while 16.7% (2/12) had moderate OHSS with abdominal distension and discomfort. Ambulatory management was efficient in both patients, even for the one who required culdocentesis. No other major complications were diagnosed.

Oocyte recipients were 37.4 ± 0.7 years old, with mean serum oestradiol levels under HRT of 306 ± 50.3 pg/ml, and endometrial thickness 8.7 ± 0.4 mm. Oocyte donation outcome was as follows:

Stimulation days	FSH stimulation dose (IU)	E2 at the beginning of coasting (pg/ml)	E2 at HCG administration day (pg/ml)	Coasting days	# Oocytes	# Oocytes metaphase II
9.8 ± 0.4	1958.3 ± 67.1	6496.9 ± 393.2	2268.6 ± 368.8	4.3 ± 0.3	23.2 ± 4.3	20.4 ± 3.8

Oocytes received	Fertilization rate (%)	Embryos transferred	Implantation rate (%)	Pregnancy rate	Abortion rate	Multiple pregnancy rate	Cancellation rate
6.8 ± 0.2	80.7	3.4 ± 0.1	23.1	16/29 (55.2%)	2/16 (12.5%)	5/16 (31.2%)	1/30 (3.3%)*

*One embryo transfer was cancelled due to very low-quality embryos.

Conclusions: Oocyte quality is not impaired in donors undergoing COH with coasting, as oocyte recipients show normal pregnancy and implantation rates compared to recipients receiving embryos from non-coasted donors. Coasting is an effective option to manage high-risk patients for OHSS, avoiding cycle cancellation without affecting oocyte donation efficiency. Although OHSS still may appear, coasting reduces its severity, allowing ambulatory management.

P-117. Experience of in-vitro fertilization surrogacy in Finland

Söderström-Anttila, V.[1], Blomqvist, T.[2], Foudila, T.[1], Hippeläinen, M.[3], Kurunmäki, H.[4], Siegberg, R.[1], Tulppala, M.[1], Tuomi-Nikula, M.[4], Vilska, S.[1] and Hovatta, O.[5]

[1]The Family Federation of Finland, Infertility Clinic, PO Box 849, 00101 Helsinki, [2]Eira Hospital, Infertility Unit, Tehtaankatu 28, 00150 Helsinki, [3]Kuopio University Hospital, Department of Obstetrics and Gynecology, PO Box 1777, 70211 Kuopio, [4]Felicitas Clinic, Urho Kekkosen katu 4–6A, 00100 Helsinki, Finland; [5]Karolinska Institutet, Department of Obstetrics and Gynecology, Huddinge University Hospital, 14186 Huddinge, Sweden

Introduction: In-vitro fertilization surrogacy (IVF-S) allows women without a functioning uterus or those with a severe medical disorder incompatible with pregnancy to have their own genetic offspring. In Finland, IVF-S treatments have been carried out at four clinics. After birth, the genetic parents adopt the child from the surrogate mother. We report our experience of all IVF-S arrangements carried out in Finland from 1991 to 2000.

Materials and methods: A total of 16 couples completed 27 IVF surrogacy cycles. Two of the couples came from Sweden, one from Norway, and one from Denmark. The mean age of the commissioning mothers was 33 years (range 20–40 years). The indications for IVF surrogacy treatment were: congenital absence of uterus and vagina (5), hysterectomy because of obstetric complications (4), hysterectomy for severe uterine disease (3), uterine abnormality (3), and severe systemic lupus erythematosus (1). The commissioning couples arranged their

surrogate mothers by themselves. One couple had two different surrogate mothers. All of them acted altruistically without commercial involvement. In 11 cases the carrier was a close relative of the commissioning couple (sister 6, mother 3, husband's sister 1, cousin 1). The mean age of the surrogate mothers was 36 years (range 29–52 years). They had successfully delivered at least one child of their own (in mean 2.6 per woman). Both the genetic mothers and the gestational carriers were medically screened and counselled by an independent psychologist. The genetic mothers were stimulated according to a long ovarian stimulation protocol. In 15 cases, the embryos were transferred in a natural cycle and in 18 cases in a hormone replacement therapy cycle. After pituitary down-regulation, the surrogate mothers used oestradiol valerate, 4–6 mg/day, and vaginally administered natural progesterone, 600 mg/day, starting 2–3 days before embryo transfer (ET).

Results: Two cycles were cancelled due to unresponsiveness of the ovaries. One couple received eight anonymously donated oocytes. An average of 13.1 oocytes (315/24) (range 1–30) were collected. The fertilization rate was 54.2% (175/323). The clinical pregnancy rate (PR)/fresh ET was 53.3% (8/15). An average of 1.8 embryos were transferred at a time. The implantation rate was 33.3% (9/27). The clinical PR/frozen–thawed embryos was 16.7% (3/18). The ongoing PR/genetic couple was 62.5% (10/16). Nine infants (7 singletons, one pair of twins) were born and two pregnancies are ongoing. One pregnancy ended in miscarriage. Caesarean section was carried out in five of eight labours (62.5%). The mean birth-weight of singleton infants was 3526 g (2270–4650 g). The birth-weight of the twins was 2900 g and 2400 g. In most cases the IVF-S arrangements worked well. As far as we know there were two cases of disagreement and unhappiness between the genetic couple and the surrogate. One of these surrogate mothers suffered from postpartum depression.

Conclusions: According to our experience, the IVF-S treatments have mostly gone smoothly without any major problems. Proper assessment of the surrogacy arrangements, thorough patient preparation, and careful counselling throughout the pregnancy and after the birth of the child are vital parts of the process and enable a high success rate and a favourable outcome for the parties involved.

P-118. Self-regulation and transfer of fewer embryos reduce multiple pregnancies after assisted conception

Lancaster P.A.L. and Hurst T.

AIHW National Perinatal Statistics Unit, The University of New South Wales and Sydney Children's Hospital, Sydney NSW 2052 Australia

Introduction: Compared with most other countries, Australia and New Zealand have achieved relatively low rates of multiple pregnancies after assisted conception. However, because these clinical services are widely available, multiple births after assisted conception have increasingly contributed to the rising rate of multiple births in the population.

Methods: We analysed trends in multiple pregnancies after assisted conception in Australia and New Zealand and compared multiple pregnancy rates for the various types of assisted conception (other IVF, fresh; other IVF, thawed; ICSI, fresh; ICSI, thawed; and GIFT). For births occurring in Australia from 1991 to 1998, we compared births after assisted conception with population data from the perinatal data collections.

Results: Among 26 713 assisted conception pregnancies of at least 20 weeks gestation conceived between 1979 and 1998, there were 4990 (18.7%) twin pregnancies, 603 (2.3%) triplet pregnancies and 37 (0.1%) pregnancies resulting in quadruplets or quintuplets. Twin pregnancies declined from more than 20% in the late 1980s to 17.1% in 1994, and then increased slightly to 18.9% in 1998. Triplet pregnancies fell from more than 3% in the 1980s to 1.3% in 1998, and higher order multiple pregnancies also declined. In the period between 1991 and 1998, multiple pregnancies were more likely after transfer of fresh embryos (ICSI, 21.5%; other IVF, 21.8%) and GIFT (25.1%) than after transfer of thawed embryos (ICSI, 13.4%; other IVF, 13.1%). Relatively fewer embryos were transferred to the uterus after cryopreservation than in fresh transfers. In Australia, births after assisted conception accounted for 1.4% of all births in 1998. The contribution of assisted conception to all twin births increased from 9.0% in 1991 to 15.3% in 1998. Assisted conception accounted for 44.9% of triplets and 55.0% of higher-order multiple births in Australia between 1991 and 1998. In 1998, the average number of embryos or oocytes transferred was less for thawed embryos (2.1) than for fresh embryos or oocytes (2.3). Multiple births accounted for 59.8% of perinatal deaths after assisted conception compared with 12.8% for all births.

Conclusions: By generally adhering since 1988 to voluntary guidelines that recommended transfer of no more than three embryos or oocytes, IVF practitioners in Australia and New Zealand have effectively reduced the incidence of multiple pregnancies, more so triplets and higher-order multiples than twins. Because fewer embryos are transferred in thaw than in fresh cycles, relatively greater use of thawed embryos will assist in reducing multiple births. On the other hand, expansion of IVF clinical services results in more births, more multiple births, and relatively more multiple births being due to assisted conception. Further reduction in multiple pregnancies after assisted conception is possible as fewer embryos are transferred.

P-119. GnRH antagonist in poor responders undergoing ART

Ferraretti A.P., Gianaroli L., Balicchia B., Iammarrone E., Bafaro G. and Magli M.C.

S.I.S.ME.R, Reproductive Medicine Unit, Bologna, Italy

Introduction: For some years our approach to poor responders after a conventional gonadotrophin-releasing hormone GnRH agonist down-regulation protocol is to perform a further stimulation treatment without GnRHa because the agonist might have a deleterious effect in these patients. However, the prognosis for these difficult cases is not statistically improved using these protocols (because of high cancellation rates and low pregnancy rates). Since GnRH antagonists are available for clinical use, we have tested their efficiency in poor responders.

Materials and methods: Our study group consisted of 49 patients classified as poor responder (cycle cancelled or $\leqslant 3$ recovered oocytes) in a previous GnRH down-regulated cycle. All of them underwent COH using 450 IU of recombinant FSH from day 2 of the cycle. Ultrasound and oestradiol monitoring were started on day 7 of the cycle. GnRH agonist in a multiple dose protocol (Cetrorelix 0.25 mg/day) was added when the leading follicle reached 15 mm in diameter and continued to the day of HCG injection (group I). Twenty-eight poor responders in the same age-range undergoing the same stimulation protocol without antagonist were used as control (group II). Oocytes retrieval, insemination, culture procedures, and embryo transfer technique were similar in the two groups.

Results: The results are shown in the Table.

	Group I	Group II
No. of cycles	49	28
No. of cancelled cycles	15 (31%)	14 (50%)
No. of retrieval	33	14
Mean retrieved oocytes	3.8	4.0
No. embryo transfer	21	5
Mean transferred embryos	1.6	1.6
No. pregnancies (PR)	9 (43%)	1 (20%)
No. miscarriages	3	1
Implantation rate (IR)	30%	12%

The embryo cleavage rate during the 3 days of culture and the implantation rate in the antagonist group were similar to a normal responders group treated during the same period with conventional GnRH agonist down-regulation protocol (386 patients, 32% PR, 24% IR).

Discussion: Our data show that GnRH antagonist might be an important tool for poor responders, confirming other similar results already published. The most important effect of the antagonist seems to be the reduction of the cancellation rate, while the number of collected oocytes was not improved compared to poor responders treated without antagonist. However, the higher implantation rate observed in the study group suggests superior competence of the recovered eggs when stimulation

management is not influenced by the risk of a spontaneous LH surge. Furthermore, the in-vitro embryo development and the high implantation rate observed in the study group seems to exclude a potential toxic effect of the antagonist at ovarian and endometrial levels.

P-120. GnRH antagonist versus GnRH agonist—is women's age a factor in choosing the appropriate protocol for IVF?

Tur-Kaspa I., Meltzer S., Pinchasov M., Katz N., Ben-Yair E. and Segal S.

IVF Unit, Department of Obstetrics and Gynecology, Barzilai Medical Center, Ben-Gurion University, Ashkelon, Israel

Introduction: Gonadotrophin-releasing hormone (GnRH) antagonists were introduced recently for pituitary down-regulation as new IVF protocols. All published studies included young women. We investigated whether age may play a role in choosing the appropriate protocol, GnRH antagonist versus agonist, for IVF treatment.

Materials and methods: One hundred and twenty-eight women, age 20–42 years, undergoing consecutive oocyte retrievals for IVF treatment from September to December 2000 participated in this study. We offered all women the new drug (i.e. the GnRH antagonist) with the information that it carries similar pregnancy rates (PR) to that of the agonist, but it shortens significantly the number of injections needed. In our present health system, the use of GnRH antagonist costs the patient about $250 more compared with the agonist, and this was a major reason for some patients to prefer one of the drugs to the other. Seventy-three patients received GnRH agonist (Decapeptyl) for downregulation, 50 were <35 years and 23 were >35 years old. Fifty-five received the GnRH antagonist (Cetrotide), in which sub-group 23 were <35 years and 17 were >35years old.

Results: Ninety per cent of women aged <35 years with agonist reached embryo transfer, compared with 92.1% with antagonist <35 years; 91.3% of agonist >35 years and only 76.5% of the antagonist >35 years group. There were no significant differences between the groups in mean age or the number of embryos transferred. The clinical PR was 29% in the group of women <35 years with the agonist, 45.7% for the antagonist <35 years, 39% for the agonist >35 years group, and 15.4% for the antagonist >35 years group.

Conclusion: The use of GnRH agonist may occasionally be accompanied by 'over' suppression and a decrease in the ovarian response, especially in women >35 years old. We therefore expected that the use of the antagonist might be more appropriate for these women. However, our initial results do not support this hypothesis. Fewer patients aged >35 years reached embryo transfer with the antagonist, and their PR was further reduced compared with the agonist group. At age <35 years, the results were comparable for both groups. Further studies are needed to examine whether a woman's age plays a role in choosing the appropriate protocol for IVF between down-regulation with GnRH antagonist or with agonist

P-121. Nitroglycerine transdermal patches during embryo transfer in patients with low uterine receptivity

Galera F., Verdú V., Villafáñez V., Garijo E. and Bajo J.M.

GINEFIV, Madrid

Introduction: Pregnancy rate appears to be diminished in patients with low blood flow and uterine receptivity. Vasodilators and their administration are being studied to help improve the pregnancy rate in these patients.

Objectives: The objective of this work was to determine the potential benefit of nitric oxide by the administration of nitro-glycerine transdermal patches in patients with low uterine receptivity, in an attempt to achieve improved pregnancy rates after embryo transfer.

Methodology: Uterine receptivity was evaluated by a scoring system from our Hospital (SEF, Seville 1999) using transvaginal Doppler. The scoring ranged from 0 to 20 points. We evaluated the homogeneity of the endometrium (0–2 points) as well as the colour map and the presence of sub-endometrial flow (0–3 points). We also checked the resistance index in the uterine artery and the absence or presence of diastolic flow in uterine, arcuate, intra-myometrial, and spiral arteries (0–4). We included patients with at least three good-quality embryos, less than 40 years of age, and with baseline levels of FSH, LH, and prolactin within normal values. Patients scoring under 10 were divided into two groups, A and B. Group A did not receive any treatment while group B received Nitraderm TTs 10 (10 mg/24 h) on the day of the embryo transfer. Patients were randomized 2:1 to groups A and B. The chi-square test was used for comparisons between study groups.

Results: No pregnancy was obtained in group A (0/18), while the rate of pregnancy through transfer was 55.5% (5/9) ($P < 0.001$). Implantation rates were 0/54 in A versus 9/27 (33.3%) in B ($P < 0.001$). The scoring increased by 25.5% from an average value of 8.4 before patch therapy to 12.9 at 24 h after the patch administration.

Conclusions: Although the number of patients included in the study was limited, there were statistically significant differences between groups in pregnancy rates after transfer and implantation. There was also a clear improvement in the receptivity index evaluated by echo Doppler. These findings justify the use of transdermal patches of nitro-glycerine in order to improve implantation rates.

P-122. Serum inhibin B levels before start of gonadotrophin treatment can predict ovarian response in combined GnRH-analogue + gonadotrophin stimulation

Urbancsek J.[1], Hauzman E.[1], Murber Á.[1], Klinga K.[2], Rabe T.[2], Strowitzki T.[2] and Papp Z.[2]

[1]First Department of Obstetrics and Gynaecology, Semmelweis University School of Medicine, Budapest, Hungary; [2]Division of Gynaecological Endocrinology, Department of Obstetrics and Gynaecology, Ruprecht-Karls University, Heidelberg, Germany

Introduction: With increasing understanding of the control of inhibin A and B production and with the possibility of the exact measurement of these two different types of the inhibin family, more attention has been given to inhibin B as a marker of ovarian response. In our retrospective analysis, we investigated whether serum inhibin B concentrations may have a predictive value in regard to ovarian response, after pituitary desensitization, but before the start of gonadotrophin treatment.

Materials and methods: Serum inhibin B concentrations of 13 IVF patients with poor ovarian response were measured after pituitary desensitization, just before the start of gonadotrophin stimulation, on the day of ovulation induction with HCG, and on the day of oocyte retrieval. Poor response was defined as number of retrieved oocytes ⩽3 and serum oestradiol concentration ⩽900 pg/ml on the day of HCG administration. Thirteen patients with normal ovarian response (no. of retrieved oocytes ⩾6, serum oestradiol on day of HCG ⩾1800 pg/ml) were taken as controls. Cases and controls were matched by age, by serum FSH concentration on day 3 of a spontaneous cycle, and by the cause of infertility. Pituitary desensitization was achieved by triptorelin (0.1 mg Decapeptyl®, Ferring, Germany) given s.c. for 10–14 days from day 20 of the previous cycle. When serum oestradiol concentration was ⩽50 pg/ml, HMG (75 IU Humegon®, Organon, Holland) or FSH (75 IU Metrodin®, Serono, Switzerland) stimulation was started with three ampoules per day for 3 days and two ampoules per day for the following 2 days. Afterwards, the amount of administered HMG was adjusted to the ovarian response. When the leading follicle was ⩾18 mm in diameter and at least two other follicles ⩾16 mm were present, and serum oestradiol concentration was ⩾300 pg/ml/follicle, 10 000 IU HCG (Profasi®, Serono, Switzerland) was given for ovulation induction, and oocyte retrieval was performed 36 h later. Serum inhibin B concentrations were measured using a solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) (Oxford Bio-Innovation, Oxford, UK). The sensitivity of the assay was 15 pg/ml, and the intra- and inter-assay coefficients of variation were <20%. Statistical analysis was performed using Wilcoxon's matched-pairs test. Data are presented as medians.

Results: There was no difference between poor responders and controls regarding the age (37 versus 37 years, $P = 0.04$), distribution of indications, body mass index (23.5 versus 22.7 kg/m^2, $P = 0.74$), serum FSH (7.6 versus 7.6 mU/ml, $P = 0.33$) and oestradiol (51.6 versus 42.0 pg/ml, $P = 1$) levels on day 3 of a spontaneous cycle, and the duration of GnRH-analogue pre-treatment (17 versus 15 days, $P = 0.44$). The number of gonadotrophin ampoules (55 versus 25 ampoules $P = 0.01$) used, and the duration of stimulation (11 versus 10 days, $P = 0.01$) with gonadotrophins were significantly higher, while serum oestradiol (618 versus 2217 pg/ml, $P = 0.001$) and inhibin B (209.6 versus 765.4 pg/ml, $P = 0.01$) concentrations on the day of HCG were significantly lower in the poor responder group. Before start of stimulation serum inhibin B concentrations were found to be significantly lower in poor responders than in their normal responder controls (11.6 versus 43.8 pg/ml, $P = 0.004$), while serum oestradiol concentrations did not differ in the two groups (20.0 versus 30.3 pg/ml, $P = 0.13$).

Conclusion: Based on the observation that serum inhibin B concentrations after GnRH-analogue desensitization and prior to gonadotrophin stimulation are lower in poor-responder IVF patients than in normal responders, we assume that serum inhibin B levels can be used as a marker of poor ovarian response in IVF treatment, and can help to identify patients requiring more gonadotrophins from the beginning of the stimulation to achieve a successful ovarian response.

Acknowledgement: This study was supported by a grant of the Alexander v. Humboldt Foundation (Bonn, Germany) to J. Urbancsek.

P-123. Correlation between serum levels of LH, oestradiol and inhibin A and inhibin B and the outcome of ovarian stimulation for IVF using pure FSH following pituitary down-regulation with mid-luteal GnRH agonist

Khalaf Y., Sheward V. and Wheeler M.

Guy's and St Thomas' Assisted Conception Unit, and Department of Endocrinology and Metabolic Medicine

Introduction: We sought to observe the influence of human pure FSH after down-regulation with a GnRH analogue on circulating levels of LH, inhibin A and inhibin B and evaluate the correlation between these levels and the outcome of ovarian stimulation.

Materials and methods: The study population consisted of 46 patients who were attending our unit for IVF treatment. All subjects were ⩽43 years of age, and their basal FSH was ⩽10 U/l. All couples were suitable for the study and agreed to take part and signed an informed consent. Serum samples were withdrawn on days 2–4, on day 10 of the pre-treatment cycle, and on day 9 of ovarian stimulation with pure FSH (recombinant or highly purified FSH) following mid-luteal down-regulation with buserelin acetate. The Chiron Diagnostics ACS was used to determine the levels of FSH, LH, and oestradiol. Inhibin A was measured using ultrasensitive Oxford Bio-Innovation Ltd kit and inhibin B was measured using standard Oxford Bio-Innovation Ltd kit. The outcome measures included the number of follicles ⩾14 mm on day 9, number of oocytes obtained 34–38 h following administration of 10 000 IU of HCG and number of embryos obtained. Regression coefficients for the correlation between each hormone measurement and each outcome measure were calculated. Student t-test was used to examine for significant differences between the hormone levels in the good and poor responders to ovarian stimulation. The institutional Ethics Committee approved of the study.

Results: Day 10 serum FSH and the difference between day 10 and day 3 levels of the same hormone showed a significant negative correlation with the number of oocytes obtained ($P = 0.04190$) and number of embryos developed ($P = 0.0082$). Day 10 serum LH and the difference between day 10 and day 3 levels of the same hormone showed a significant positive correlation with the number of follicles ($P = 0.0108$) and ($P = 0422$) respectively. Day 3 and day 10 inhibin A, inhibin B, and oestradiol did not show any significant correlation with any of the outcome measures. Day 9 levels of the same hormones showed a highly significant positive correlation only between the levels of inhibin A and inhibin B and oestradiol and all outcome measures, and significant differences were noted between the good and poor responders in the three hormones. Interestingly, day 9 FSH was not correlated with the outcome whereas day 9 LH levels demonstrated a significant negative correlation with the number of embryos obtained ($P = 0.02$).

Conclusions: Basal levels of FSH, LH, oestradiol, inhibin A, and inhibin B are not significantly correlated with the outcome of ovarian stimulation for IVF. Their levels following clomiphene challenge test (day 10) were significantly correlated with the outcome of ovarian stimulation for IVF. Furthermore, following 9 days of stimulation, levels of oestradiol, inhibin A, and inhibin B are significantly correlated with the outcome, while day 9 LH level showed a negative correlation. The mechanism of increased levels of LH following stimulation with pure FSH under pituitary down-regulation conditions and their significant negative correlation with the outcome warrant further elucidation.

P-124. Preliminary experience with cytoplasmic transfer in patients with multiple in-vitro fertilization failures

Massaad Z.C., Khan I., Ghutmi W., Lakkis D., Nakhle T. and Fakih H.

IVF Michigan, Michigan State University, Rochester Hills, MI, USA

Introduction: Women presenting with multiple in-vitro fertilization (IVF) failures present an especially difficult challenge to reproductive endocrinologists. Cytoplasmic transfer has recently been described as a potential solution to overcome this problem.

Materials and methods: Women presenting with multiple IVF failures were recruited and offered cytoplasmic transfer as an adjunct to traditional IVF during the next assisted reproduction cycle. The metaphase II oocytes of recipients were injected with their husband's spermatozoa and cytoplasm aspirated from either fresh donor oocytes or cryopreserved–thawed tripronucleate zygotes of donors.

Results: Fifteen women were entered in the study; overall pregnancy rate was 6/15 (40%). Four of these (27%) resulted in live births, all with normal karyotypes. All pregnancies occurred in women less than 40 years of age ($n = 12$). The pregnancy rate was higher in the subgroup of women under 40 receiving fresh cytoplasm (5/8 pregnant; 4/8 live births) when compared to those receiving cytoplasm from cryopreserved tripronucleate zygotes (1/4 pregnant; 0/4 livebirths). All women ($n = 3$) over 40 received fresh cytoplasm but failed to conceive.

Conclusions: Cytoplasmic transfer may be a solution for women under 40 years with multiple prior IVF failures; cytoplasm from fresh donor oocytes may prove superior to that from cryopreserved tripronucleate zygotes. Further studies are needed to confirm these findings.

P-125. The influence of endometrial thickness on assisted conception treatment outcome

Rutherford A.J., Naeem T., Salha O. and Balen A.

Department of Reproductive Medicine, Clarendon Wing, Leeds General Infirmary, Leeds LS2 9NS, UK

Introduction: The disappointingly low implantation rates after replacement of morphologically normal embryos is still a major enigma in assisted reproduction treatment (ART). A few studies have investigated the influence of endometrial development on the success of ART and have demonstrated conflicting results. The objective of this study was to evaluate the relationship between endometrial thickness measured on the day of HCG administration and cycle outcome.

Materials and methods: Patients who had in-vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) treatment between January 1998 and April 2000 were included in this retrospective analysis. The Unit's computerized database was used to retrieve cycle details. All patients had the same long-protocol down-regulation process. The dose of gonadotrophins prescribed was dependent on the patients' age, body mass index, and previous ovarian response (if applicable). Tracking of follicular development was performed using transvaginal ultrasonography

and the mean maximal follicular diameter (MMFD) was calculated from four measurements in two orthogonal planes. When there were at least three follicles with a MMFD of ~18 mm, HCG 10 000 IU was administered intramuscularly. All patients were routinely scanned on the day of HCG administration and the endometrial thickness was documented. Transvaginal ultrasound-guided oocyte retrieval was performed 35–36 h post-HCG administration. Embryo transfers were performed 48–52 h post-oocyte recovery when the majority of embryos were at the 2- or 4-cell stage.

Results: In this retrospective study we analysed 206 IVF and ICSI treatment cycles. Patients were matched for age, duration of infertility, and the mean number of embryos replaced in each cycle (Table 1). Cycles were divided into three groups according to endometrial development. Patients with endometrial thickness of <10 mm had a significantly lower implantation, clinical pregnancy, and live birth rate per cycle compared with those with endometrial development of >12 mm. Similarly, patients with an endometrial development of 10–12 mm also had a better cycle outcome compared with their counterparts with an endometrium of <10 mm, although the difference did not reach statistical significance.

Table 1. The relationship between endometrial thickness on the day of HCG administration and cycle outcome

Endometrial thickness	<10 mm (n = 62)	10–12 mm (n = 92)	>12 mm (n = 52)
Age (years)	31.9 ± 3.4	31.3 ± 3.6	31.4 ± 3.0
Duration of infertility (years)	4.3 ± 2.4	4.6 ± 2.8	5.1 ± 2.8
Embryos replaced per cycle	2.0 ± 0.6	2.0 ± 0.7	2.0 ± 0.6
Implantation rate per cycle (%)	14.1	20.5	25.5
Clinical pregnancy rate per cycle (%)	22.6*	35.9	46.2*
Live birth rate per cycle (%)	21.0¶	26.1	42.3¶

Values are mean ± standard deviation. * and ¶ significant difference ($P < 0.05$).

Conclusions: In spite of much progress in assisted reproductive treatment, including improvements in ovulation induction, oocyte retrieval, and culture medium, the maximum conception rate still rarely exceeds 30% per cycle, and up to 90% of apparently normal embryos fail to implant. It is evident from this study that the development of an adequate receptive endometrium is crucial in enhancing embryo implantation and clinical pregnancy rates.

P-126. Multivitamin supplements are associated with an increase in follicular fluid antioxidant levels and appear to improve outcome in assisted reproduction

Matthews S.J.[1], White K.L.[2], Sutcliffe A.E.[2], Parsons W.J.[3], Rutherford A.J.[1], Sharma V.[1], Picton H.M.[1] and Hay A.W.M.[2]

[1]Academic Unit of Paediatrics, Obstetrics and Gynaecology, [2]Molecular Epidemiology Unit, [3]Department of Research and Development, University of Leeds, Leeds, UK

Introduction: It has been shown that antioxidants are present in human follicular fluid. The factors that influence the concentration of antioxidants in the Graafian follicle and the optimum levels required to support the production of a fertile oocyte have yet to be clarified. This study examined potential factors that might affect follicular fluid antioxidant concentrations, and assessed the impact of these levels on the outcome of assisted reproduction.

Materials and methods: Follicular fluid was collected from 215 women having oocyte retrieval. Treatment parameters and medical history were recorded for each couple. All women completed a questionnaire about general diet, smoking status, and the use of vitamin supplements. Vitamin C levels were analysed by HPLC. Similarly, vitamin A and E levels were measured on follicular fluid from 90 patients. The total antioxidant potential (TAP) was measured using enhanced chemiluminescence. All results were correlated with treatment parameters and the information from the questionnaires. Statistical analysis was performed as appropriate.

Results: Vitamin C levels were positively correlated with age (P = 0.016) and negatively correlated with increasing body mass index (BMI) (P = 0.005). There was no correlation between vitamin C levels or TAP and cause of infertility, duration of infertility, gonadotrophin dose, egg retrieval ratio, number of oocytes collected, fertilization and cleavage rates, or dietary fruit or caffeine intake. Smokers had significantly lower follicular fluid vitamin C and TAP levels than non-smoking women (P = 0.017). Vitamin supplementation was associated with increased TAP, and in higher follicular fluid levels of vitamins C (P = 0.001), and vitamin E (P = 0.033) but not vitamin A (P = 0.197). Although the pregnancy outcome was better in women with higher levels of follicular fluid vitamin C and E, the improvement is not significant. Women taking supplements did, however, have a significantly higher pregnancy rate [24/62 (39%) versus 26/138 (18.8%); P = 0.004]. When this cohort was further analysed to examine the effect of taking a vitamin C supplement only, as opposed to a combined preparation with other antioxidants including vitamin E, and compared with women who took no dietary supplements, a highly significant improvement in pregnancy rates was seen in the group on the multivitamin preparation (P = 0.0185).

Conclusions: The finding that older women have higher follicular fluid levels of vitamin C may reflect their tendency towards a healthier diet and an increased likelihood of taking vitamin supplements. The lower levels in obese women may be an indication of poor diet, but are more likely to reflect an increased requirement in this group of patients. Smokers have significantly lower follicular fluid vitamin C levels. Decreased absorption and increased metabolism of ascorbate is thought to be responsible for the well-documented decrease in plasma vitamin C in smokers, but this has never been detected in follicular fluid before. If smokers are unable to stop smoking during their treatment, they may benefit from a vitamin supplement. Higher fluid levels of vitamins C and E are associated with taking vitamin supplements. The significant improvement in pregnancy rates in women on multivitamins may reflect the influence of other components in the preparation, or the synergistic action of a combination of vitamins and minerals. These data suggest that taking a multivitamin preparation during ovarian hyperstimulation may improve IVF outcome.

P-127. Prolonged coasting (>5 days) does not decrease implantation and clinical pregnancy rates, whilst reducing the risk of severe ovarian hyperstimulation syndrome (OHSS)

Ferrara I., Balet R., Smith S., Foad F. and Grudzinskas J.G.

The Bridge Centre, One St Thomas Street, London SE1 9RY, UK

Objective: To determine whether, in women undergoing controlled ovarian hyperstimulation (COH) during IVF-ET and at risk of ovarian hyperstimulation syndrome (OHSS) prior to oocyte retrieval, withholding gonadotrophins for more than 5 days (6–11 days) will interfere with the implantation and the clinical pregnancy rates whilst reducing the risk of severe OHSS.

Design: Retrospective analysis in a tertiary care fertility centre.

Patients: Seventy patients who underwent COH per IVF-ET were at risk of OHSS. Human menopausal gonadotrophin (HMG) was withheld (coasting) for more than 5 days (7.1 ± 1.5 day, range 6–11 days) in 24 patients, while 46 were coasted for a mean of 4.0 ± 0.8 days (range 3–5 days), HCG being administered after serum oestradiol levels decreased to a 'safe' level of 10 000 pmol/l.

Results: All the patients underwent oocyte retrieval. The mean number of oocytes collected was 10.5 ± 4.7 (2–21) for patients coasted for <5 days and 6.8 ± 5.3 (0–20) for patients coasted for >5 days ($P < 0.005$). The fertilization rate was 53 and 56% (NS), the cleavage rate being 82 and 67% (NS), the implantation rate 15 and 24% (NS), and the clinical pregnancy rate was 27 and 30% (NS) respectively. In two patients coasted for more than 5 days, no oocytes were retrieved, while two women in the same group developed severe OHSS.

Conclusion: This retrospective analysis indicates that coasting over a prolonged period of 6 days or more is associated with high implantation and clinical pregnancy rates. Thus, FSH/HMG may be withheld for up to 11 days, apparently without negatively affecting treatment outcome, whilst reducing the incidence of severe OHSS.

P-128. The significance of biochemical pregnancies in assisted reproductive technology

Cuneo S., Fortini D., Magli M.C., Crippa A., Ferraretti A.P. and Gianaroli L.

S.I.S.ME.R. Reproductive Medicine Unit, Bologna, Italy

Introduction: During recent years the rate of biochemical pregnancies (BP), defined as the presence of at least two positive beta fractions of human chorionic gonadotrophin in serum, without evidence of a gestational sac at ultrasound, has been reduced dramatically in assisted reproductive technology (ART), probably because of the development of improved clinical and laboratories protocols. The aim of this study was to evaluate the current significance of the biochemical pregnancies that still occur in ART.

Materials and methods: From January 1997 to December 1999, 1713 assisted reproductive technology cycles were reviewed, to select the total cycles that developed BP ($n = 42$) and evaluate the age of the patients, the cause of infertility, the induction of ovulation protocol, the insemination technique, if a pre-implantation genetic diagnosis was performed, the embryo quality, the day of transfer, and further results in patients undergoing a second cycle. Transfers of frozen embryos and oocyte donation were excluded. The control group (157) consisted of cycles without pregnancy during the first ART treatment. The insemination techniques and pre-implantation genetic diagnosis cycles were equally distributed.

Results: Of 1034 embryo transfers: 416 (40.2%) patients became pregnant, 11 (2.6%) had an ectopic pregnancy, 36 (8.6%) ended in abortion, 327 (78.6%) had a full-term pregnancy, and 42 (4.06%) patients developed BP during the first ART treatment. Of the 42 BP, 18 underwent a second cycle: 11 (61.1%) became pregnant, one (5.5%) developed a first trimester abortion, one (5.5%) had an ectopic pregnancy, eight (44.4%) went on to full-term pregnancy, and one (5.5%) had a repeat BP. The results are shown in Table 1.

The other parameters analysed did not show statistical differences against the control group.

Table 1.

	Biochemical group	Control group	*P*
Number of cycles	42	157	–
Age	34.52 ± 4.51	34.30 ± 4.79	NS
Pregnancy in subsequent ART (%)	11 (61.1)	41 (26.1)	0.005

NS, Not significant.

Conclusions: An elevated percentage of biochemical pregnancies are probably related to a failure of the ART. Nevertheless the low cases of BP that are still present in ART seems to have a positive possibility of developing a clinical pregnancy during the following cycles. More data are needed to establish whether this trend is maintained.

P-129. The effect of combined antibacterial, anti-inflammatory and antioxidant treatment of male patients on ICSI outcome

Wasilewski T.[2], Kuczyński W.[1,2], Grygoruk C.[1,2], Pietrewicz P.[2], Czech R.[2], Wołczyński S.[3], Domitrz J.[3], Grochowski D.[3], Szamatowicz M.[1]

[1]Department of Gynaecology, Medical University of Białystok; [2]Infertility Centre Cryobank, Białystok, Poland; [3]Department of Gynaecological Endocrinology, Medical University of Białystok, Poland

Objective: To evaluate the effect of antibacterial, anti-inflammatory, and antioxidant treatment of male patients on the intracytoplasmic sperm injection (ICSI) outcome.

Materials and methods: Fifty-six subfertile men (group I), diagnosed as having oligoasthenozoospermia and asymptomatic leukocytospermia (WHO), were treated using a combination of antibacterial drugs (co-trimoxazole 960 mg/day for 20 days), anti-inflammatory drugs (piroxicam 20 mg/day for 20 days, then methylprednisolone 40 mg i.m., in a single dose on the 40th day of therapy), and antioxidant drugs administered for 90 days: vitamin A 36 000 IU/day, vitamin E 600 mg/day, vitamin C 800 mg/day, co-enzyme Q 30 mg/day, pentoxifylline 400 mg/day. The control (group II) consisted of 244 patients undergoing planned ICSI treatment. The results of controlled ovarian hyperstimulation (COH), fertilization rate, early embryo development parameters, and clinical pregnancy rates were taken into consideration.

Results: The mean age, past medical history concerning infertility factors, and the mean serum oestradiol levels between the examined groups were not significantly different. In group I, 85.4% of obtained oocytes were MII, as against 87.2% in group II. The fertilization rate expressed as a proportion of 2PN oocytes per injected MII oocyte was 49.8% in group I and 54.9% in the control group ($P > 0.05$). Two days after ICSI, the percentage of embryos at the 4-cell stage was 71.9% in group I and 80.6% in group II ($P > 0.05$). Nevertheless, the percentage of good-quality embryos in group I was significantly higher in comparison to group II. The pregnancy rate in group I was 33.9% per started cycle and 35.2% per transfer, while the pregnancy rate in the control group was 22.6% per started cycle and 23.0% per transfer.

Conclusion: The treatment of subfertile men with combined antibacterial, anti-inflammatory, and antioxidant medication might be beneficial to ICSI outcome. Nevertheless, further investigations on this subject are needed.

P-130. Artificial endometrial preparation for frozen–thawed embryo transfer using oral oestradiol and a new low-dose vaginal progesterone preparation: Endometrin tablets

Lewin A., Fatum M., Shufaro Y., Simon A., Reubinoff B., Laufer N. and Safran A.

IVF Unit, Dept of Obstetrics and Gynecology, Hadassah Medical Center, Hebrew University, Ein-Kerem, Jerusalem 9112D, Israel

Introduction: There are various successful protocols for artificial endometrial preparation comprising induction of endometrial proliferation with oestrogens and secretory transformation with progestogens. The aims of this prospective study were: (1) to evaluate the endometrial sonographic as well as histological preparation using sequentially oral oestradiol and a new low-dose vaginal natural progesterone preparation (Endometrin vaginal tablets), (2) to evaluate the preferred oestradiol preparation

Materials and methods: Twenty-one patients expecting frozen–thawed embryo transfer in our IVF programme were enrolled in this study, following informed consent, and were divided randomly into two groups. Both groups received oral oestradiol tablets from the beginning of menstruation, group A (11 patients) receiving 4 mg/day in two doses of 2 mg each, and group B (10 patients) received 6 mg/day in three doses. The mean patient age was similar in both groups (32.8 ± 5.5 years in group A and 31.8 ± 6.7 in group B), and the causes of infertility were also similar. Serum oestradiol, progesterone, and sonographic thickness of the endometrium were measured every 5 days throughout the mock cycle. About day 10, with endometrial thickness of ⩾8 mm, Endometrin vaginal tablets were added at a dose of 200 mg/day in two doses. Following the later 10 days of oestradiol + progesterone, an endometrial biopsy was taken using Pipelle from each patient.

Results: In all 21 patients appropriate changes in oestradiol, progesterone, and endometrial thickness were observed. In one patient in group B an out-of-date endometrium of day 18–19 on day 23–25was noted.

Conclusions: Appropriate secretory transformation induced by oestradiol and a low dose of progesterone vaginal tablets (Endometrin 200 mg/day) was observed in most patients (20 of 21). A low dose of oral oestradiol should preferably be used, as the higher oestrogenic effect may be a difficult counterpart to the low progesterone dosage.

P-131. The prognostic significance of day 3 embryo cleavage stage on subsequent blastocyst development

Boostanfar R., Jain J.K., Slater C.C., Tourgeman D.E., Francis M.M. and Paulson R.J.

Department of Obstetrics/Gynecology, University of Southern California Keck School of Medicine, Los Angeles, CA, USA

Objectives: To determine the prognostic significance of the number of blastomeres observed on day 3 of embryo culture upon subsequent development to the blastocyst (BL) stage.

Design: A retrospective review of patients undergoing IVF with BL transfer.

Materials and methods: During 1999, 37 cycles with BL transfer were identified. All subjects were under the age of 35 years and underwent standard ovarian hyperstimulation with leuprolide down-regulation followed by HMG or FSH stimulation. Embryos were cultured in 50-µl drops of media under oil and grown in sequential P1 and BL media. Embryos were cultured until day 5 or day 6 and were evaluated for transfer or cryopreservation.

Results: A total of 763 oocytes were obtained, of which 431 fertilized (56.5% fertilization rate). The total number of BL noted on days 5 and 6 of culture was 243. The percentage of BL per oocytes aspirated was 31.9% and per fertilized oocyte was 56.4%. Table 1 shows the data comparing the cell stage on day 3 and subsequent BL development on day 5 and day 6. Of day 3 embryos that contained six blastomeres or less, 34.7% (58/167) developed into BL. Of day 3 embryos that contained seven or more blastomeres, 68.9% (186/270) progressed to BL, $P < 0.0001$. Whereas 68.8% (167/243) of BL were observed on day 5, extended culture of embryos to day 6 resulted in additional BL, representing a substantial proportion of total BL: 31.2% (76/243).

Table 1.

Day 3	Blast on day 5	Blast on day 6	Total blast, days 5+6
2 cell	0% (0/5)	0% (0/5)	0% (0/5)
3 cell	7.0% (1/15)	7.0% (1/15)	13.3% (2/15)
4 cell	18.2% (8/44)	27.2% (12/44)	45.5% (20/44)
5 cell	23.5% (8/34)	11.8% (4/34)	35.3% (12/34)
6 cell	14.4% (12/69)	14.4% (12/69)	34.8% (24/69)
7 cell	50.0% (15/30)	23.3% (7/30)	73.3% (22/30)
8 cell	47.8% (90/188)	17.0% (32/188)	64.5% (122/188)
9 cell	66.6% (2/3)	0% (0/3)	66.6% (2/3)
10 cell	63.9% (30/47)	17.0% (8/47)	80.1% (38/47)
⩾12 cell	100% (2/2)	–	100% (2/2)

Conclusions: (1) The presence of seven or more blastomeres in a cleavage-stage embryo on day 3 is associated with a significantly greater likelihood of developing to BL stage on day 5 or day 6 of culture. (2) Embryos that contain four, five, or six blastomeres on day 3 are still associated with a relatively good likelihood of blastocyst development (38.1%). (3) Extended culture of embryos to day 6 still yields a significant proportion of blastocysts. (4) Cell cleavage stage on day 3 appears to be a good prognostic indicator of subsequent blastocyst development.

P-132. Effects of sperm placement during intracytoplasmic sperm injection (ICSI)

Iaconelli A. Jr.; Rossi-Ferragut L.M., Aoki T., Rocha C.C., Medeiros A.R.C., Santos D.R., Pasqualotto F.F. and Borges E. Jr.

Fertility-Assisted Reproduction Center, Avenida Brigadeiro Luis Antonio, 4258 São Paulo, Brasil

Introduction: When performing intracytoplasmic sperm injection (ICSI) on human oocytes, the injection is normally made at the 3 o'clock position, with the first polar body (PB) at the 12 or 6 o'clock position. This orientation stabilizes the presumed spindle position furthest from the path of the injection needle and is aimed at minimizing the potential for spindle damage. However, it is still unclear whether there is an optimal area within the oocyte to deposit the sperm relative to the location of the meiotic spindle. For these reasons, this prospective study was conducted to evaluate the effect of sperm placement relative to the MII spindle location on fertilization, cleavage, and pregnancy rates.

Materials and methods: A total of 143 couples underwent 175 ICSI cycles from March to September 2000. Following follicular aspiration and oocyte retrieval, the oocytes and sperm preparations were cultured according standard protocols. All micromanipulations were performed with fresh oocytes in metaphase II, according the technique described by Palermo (1992), with the exception that oocytes were rotated into two distinct orientations so that the first polar body was stabilized at 7 or 6 o'clock. The injection pipette was always inserted into the oocyte at the 3 o'clock position. Oocytes were cultured individually in microdroplets. Eggs were examined for fertilization at 16–20 h after ICSI. Normal fertilization was defined by the presence of two distinct pronuclei and two polar bodies. Embryonic development was assessed on day +3 culture. For the purpose of this study, high-quality embryos were those having at least 6 blastomeres, at most 20% fragments, and no apparent morphological anomalies. Statistical analysis was performed with one-way analysis of variance, following by Student *t*-test for multiple comparisons. $P < 0.05$ was considered significant.

Results: The mean female age (±SD) was 35.4 ± 5.7 years. Most transfers were mixed, with both 7 and 6 o'clock embryos ($n = 104$/65.4%). Only 5% of total of transfers were non-mixed with only 6 o'clock embryos. On the other hand, 29.6% ($n = 47$) of transfers were non-mixed with only 7 o'clock embryos. Interestingly, 100% total clinical pregnancies were achieved when at least one 7 o'clock embryo was transferred. Another results are shown in the Table below.

	7 o'clock	6 o' clock	Total
Micromanipulated oocytes	785	596	1381
Intact oocytes	698	512	1210
Embryo number	622	442	1064
Normal fertilization (2PN)	501 (80.5%)	349 (79%)	
Fertilization failure	76 (10.9%)	93 (13.7%)*	
Cleavage rates	560 (90%)	375 (85.1%)	
High-quality embryos	433 (69.6%)	271 (61.3%)	
Embryo transfer total number	333 (53.5%)	195 (44.2%)	
Non-selected embryos	174 (28%)	136 (30.8%)	
Total pregnancy rate/cycle			16.60%
Total pregnancy rate/patient			20.40%
Implantation rate			17.10%

*$P < 0.05$.

Conclusions. We found better embryo quality when ICSI was performed with the polar body at 7 o'clock, even though no significant differences were found in the majority of aspects analysed. Because embryo transfers were mixed, we could not analyse the particular embryo implantation potential. However, clinical pregnancy was achieved in 100% of cycles with at least one embryo obtained by ICSI in the 7 o'clock position. Indeed, these embryos have shown higher than normal cleavage status and they will probably optimize implantation rates, offering better embryos with less damage to the MII spindle.

P-133. Blastocyst transfer after vitrification in a hemi-straw (HS) system

Vandervorst M.[1], Vanderzwalmen P.[1], Standaart V.[1], Debauche C.[1], Bertin G.[1], Zech H.[2], Zech I.[2], Bollen N.[1], van Roosendaal E.[1] and Schoysman R.[1]

[1]Schoysman Infertility Management Foundation (SIMAF), Vaartstraat 42, 1800 Vilvoorde, Belgium; [2]Institut fur In vitro Fertilisierung, Bregenz, Austria

Introduction: For several years vitrification of blastocysts in 0.25 ml French mini-straws has been considered an attractive cryopreservation option. When plunging the sealed straw into liquid nitrogen, the highest cooling rate was limited to approximately 2500°C/min. However, the success of vitrification could be improved by increasing the cooling rate up to 20 000°C/min. Different promising vitrification methods allowing direct contact between the embryos and liquid nitrogen were developed (open pulled straws, electron-microscope copper grids, nylon loops system). However, the handling or storage of these vitrified cryopreserved

blastocysts remains impractical. We developed a modified straw system, allowing direct contact between the blastocysts and liquid nitrogen in order to facilitate storage. We present our preliminary clinical data after vitrification of blastocysts using a hemi-straw (HS) system.

Materials and methods: In 24 cycles, blastocysts were vitrified using the HS procedure. A solution of ethylene glycol (EG) and DMSO was used for the vitrification. The blastocysts were first equilibrated for 2 min in EG (10%)–DMSO (10%) and subsequently immersed in EG (20%)–DMSO (20%). Next the blastocysts were suspended on a thin film on the HS, after which the HS was immersed into liquid nitrogen and finally loaded and sealed in a larger straw of 500 ml. Before thawing, the straws were suspended in sucrose before dilution into medium.

Results:

Vitrification cycles (*n*)	24
Embryos (*n*)	59
Intact embryos after thawing (*n*)	46 (78%)
Transferred embryos (*n*)	43
Transfer rate	24 /24 (100%)
Ongoing pregnancy (%)	7 (3 twin) (29%)
Ongoing implantation rate	10/43 (23%)

Conclusion: Our preliminary data demonstrate that vitrification using the hemi-straw system is a convincing cryopreservation method for blastocysts allowing a very fast cooling rate and easy storage. Contact between liquid nitrogen and the cryoprotectant may be a source of contamination, and the HS technique needs to be refined in order to reduce such potential risk.

P-134. Success of IVM/ICSI programme in PCOS patients: first IVM/ICSI baby from Turkey

Enginsu M.E., Kolankaya A., Çaml1bel T.F., Sar1kam1_ B., Kurt E., Yıldız K. and Hünerli Ç.

Metropolitan Florence Nightingale Hospital, Assisted Reproductive Technologies Department, Gayrettepe Mahallesi Cemil Aslan Güder sokak No. 8 Beşiktaş-İstanbul, Turkey

Polycystic ovary syndrome (PCOS) is a common cause of infertility. PCOS patients in assisted reproduction technology (ART) programmes have a higher risk of ovarian hyperstimulation syndrome, which can be a life-threatening problem. The monitoring of PCOS patients in controlled ovarian hyperstimulation (COH) cycles is therefore very complicated. The aim of this study was to collect and mature oocytes *in vitro*, from the natural cycle of PCOS patients prior to intracytoplasmic sperm injection (ICSI)/embryo transfer.

Materials and methods: Seven PCOS patients with histories of either previous hyperstimulation syndrome or cycle cancellation due to poor response were included in this study. All patients were examined with vaginal USG to exclude any follicular growth greater than 12 mm, from cycle day 8 to cycle day 12. Human chorionic gonadotrophin (HCG) priming was carried out between cycle days 10 and 12 with 10 000 IU Pregnyl (Serono). Starting from the day of oocyte retrieval, patients received oestradiol valerate 3×2 mg/day orally until the end of the 6th week of pregnancy. Luteal-phase support was done with 50 mg progesterone in oil i.m. daily until the 10th week of pregnancy. Oocyte retrieval was performed at 36 h following the HCG injection, from all visible follicles via a 17-gauge single-lumen aspiration needle (Cook Australia). The aspirates were examined and cellular masses examined to detect oocytes. Oocytes were then transferred into TC-199 medium (Sigma) supplemented with Na pyruvate, HCG (Pregnyl), human menopausal gonadotrophin (HMG) (Pergonal, Organon), and HSA. Sperm was collected on the day of insemination. After a culture period of 24–36 h, matured oocytes were denuded and ICSI applied. Injected oocytes were transferred into G1.2 medium (Scandinavian Science). Embryo transfer was carried out in G2.2 medium (Scandinavian Science) with Wallace transfer catheters.

Results: Of 50 cumulus corona complexes with a visible GV oocyte, 42 (84%) showed a first polar body, indicating maturation following 24–36 h of incubation in in-vitro maturation medium. Fertilization and cleavage rates were within acceptable limits. Two patients became pregnant; one delivered a healthy boy and the other has a healthy 3rd-trimester pregnancy.

Number of patients	7
Number of oocytes collected	50
Number of oocytes matured *in vitro*	42 (84%)
Number of oocytes fertilized	32 (76%)
Cleavage rate per 2 PN	88%
Mean number of transferred embryo	2.9
Implantation rate	10%
Clinical pregnancy (%)	2 (29%)

Discussion: Hyperstimulation syndrome is a major dilemma for the clinician, since its occurrence cannot be disregarded. Close monitoring of PCOS patients with serial oestradiol and USG screening is also a burden. This pilot study therefore suggests the use of IVM/ICSI cycles in PCOS patients who already have multiple follicular development potential, by making close monitoring unnecessary and eliminating hyperstimulation. In addition, this procedure decreases the cost of ART by reducing drug expenses.

P-135. Predictors of ovarian reserve in IVF/ICSI; the role of embryo quality and quantity

Wetzels A.M.M.[1], Smeenk J.M.J.[1], Thomas C.M.G.[2], Haverkate D.[2] and Braat D.D.M.[1]

Departments of [1]Obstetrics and Gynaecology and [2]Chemical Endocrinology, University Medical Centre St Radboud Nijmegen

Introduction: The ovarian reserve can be described as the size and the quality of the remaining follicle pool in the ovaries. Several biomedical predictors of ovarian reserve have been identified in relation to the results of in-vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). Overall, the quantity of oocytes was often taken into account as the main measure of the ovarian reserve. Embryo characteristics could provide additional information in determining the ovarian reserve. In a prospective study, we compared known predictors of ovarian reserve in relation to the number and quality of the generated embryos.

Materials and methods: In a prospective study 172 patients participated. Only first treatment cycles were studied. The ovaries were stimulated in a long protocol with Decapeptyl® and Puregon®. Basal (cycle day 3, prior to down-regulation) FSH, inhibin B, number of antral follicles (2–10 mm), and ovarian volume were assessed. Ovarian response, number of embryos, quality of the transferred embryos and ongoing pregnancy rates were outcome measures. Poor response was defined as <4 follicles on transvaginal ultrasound. Embryo quality was defined as a score in which the number of cells was multiplied by the morphological score (ascending scale 1–4, in which general aspect and percentage of fragmentation were considered). Age of the woman and nature of the cycle, IVF versus ICSI (based upon semen parameters), were included in the analysis. The data were analysed using logistic regression.

Results: Seventeen cycles were stopped as a result of poor response, no fertilization occurred in 17 cycles, 138 women went through embryo transfer (79 IVF cycles and 59 ICSI cycles). Low inhibin B ($P = 0.01$) and small numbers of antral follicles ($P = 0.03$) were negatively associated with poor response; other factors, including FSH, were not significantly related. Higher levels of basal FSH, however, were found to be negatively associated with the quality ($P = 0.03$) and quantity ($P = 0.05$) of embryos. ICSI was positively associated with embryo quantity, which was due to better fertilization rates compared with IVF. The quality-score of the transferred embryos was both positively correlated with the number of embryos available ($r = 0.32$, $P < 0.1$) and ongoing pregnancy rates ($r = 0.22$, $P < 0.01$). Pregnancy rates, however, had a much lower association ($r = 0.02$, $P = 0.81$) with the number of available embryos. However, no significant relationship between any of the pre-treatment variables and ongoing pregnancy rates could be found.

Conclusions: Basal inhibin B and ovarian follicle count were found to be associated with poor response. Basal FSH was found to be significantly associated with the quantity of the available embryos and the quality of

the transferred embryos. No pre-treatment predictors of pregnancy could be identified.

P-136. Anonymous oocyte donation programme based on permutation of related donors and oocyte sharing

Delbaere A., Van den Bergh M., Emiliani S., Collette M., Govaerts I. and Englert Y.

Fertility Clinic, Erasme Hospital, Free University of Brussels, Belgium

Introduction: Oocyte donation represents a treatment modality for a broad spectrum of infertility conditions. The aim of this study was to assess the success rate of an anonymous oocyte donation programme based on oocyte sharing with permutation of related donors.

Materials and methods: From January 1990 to December 2000, all consecutive egg donation cycles were retrospectively analysed. Sixty-one egg donation cycles were matched with 278 recipient cycles corresponding to 108 different patients. Fifty-eight per cent of the recipients were pre-menopausal at the time of the treatment, indications for oocyte donation being recurrent failure of IVF treatment ($n = 55$) or genetic factors ($n = 8$). Forty-two per cent of the recipients were menopausal due to idiopathic premature ovarian failure ($n = 17$), Turner syndrome or Turner mosaic ($n = 11$), other genetic causes ($n = 2$), ovarian dysgenesis ($n = 3$), chemotherapy and/or radiotherapy ($n = 5$), surgical removal of the ovaries ($n = 6$), and age ($n = 1$).

Results: Mean donor age was 29.9 ± 4.1. A total of 1022 oocytes were retrieved (mean of 16.7 per oocyte retrieval). Six hundred fifty-seven embryos were obtained (fertilization rate:64%), from which 434 were transferred in recipient fresh cycles. Seventy embryos were frozen, 25 of which were subsequently thawed and 10 transferred. Mean recipient age was 36.0 ± 5.7. Sixty recipient cycles were abandoned (40 because of an absence of oocytes), so that 218 recipients cycles received at least one oocyte. Embryo transfer was performed in 193 fresh recipient cycles (mean of 2.2 embryos by transfer) and in 10 frozen recipient cycles. Fifty-five pregnancies were obtained (one from a frozen embryo) of which 46 were clinical and nine biochemical. Thirty-six patients delivered (12 twins and one triplet), four pregnancies are still ongoing in the second or third trimester, four pregnancies ended in miscarriages and two in ectopic pregnancies. The global pregnancy rate per embryo transfer was thus 28%, and was 90% per oocyte retrieval.

Conclusions: Sharing the oocytes between different recipients following a pre-established distribution plan allows a high yield per donor, with 90% pregnancy by donor oocyte retrieval. Consequently, this system reduces the global risks related to the procedure for the donor. This is mainly related to the paucity of supernumerary embryos, minimizing the use of frozen embryos.

P-137. Empty follicle syndrome (EFS): new way of prediction and management

Lipowicz R., Albamonte M., Grabia A., Brugo Olmedo S., Papier S., Chillik C. and Fiszbajn G.

Centro de Estudios en Ginecología y Reproducción (CEGyR), Viamonte 1438 (1055), Buenos Aires. Argentina

Introduction: We define empty follicle syndrome (EFS) as the absence of mature oocytes and granulosa cells at aspiration, despite a normal ovarian response to stimulation with gonadotrophins under GnRH agonists suppression. We describe our experiences with EFS and propose new ways to manage and prevent it.

Materials and methods: We divided our experience in three periods. In the first one, between June 1994 and November 1995, we observed nine cases of EFS, of which in the first five cycles all follicles were aspirated. In the other four cases, only one ovary was aspirated and as soon as the embryologist made the diagnosis of EFS, aspiration was discontinued. At that time human chorionic gonadotrophin (HCG) determination in plasma was negative. A new dose of 10 000 IU of HCG was given, and aspiration of the other ovary was performed 34 h later. In the second period, between December 1995 and August 1998 and based in our previous experience, we decided to do urinary or serum β-HCG concentration 16 h after HCG administration. We observed only two cases in this period, in whom, although they had doubtful urinary determination or low positive serum β-HCG value, we decided to perform follicular aspiration; this procedure was interrupted when the embryologist made a diagnosis of EFS. At that stage a new dose of HCG was given and aspiration of the other ovary was performed 35 h later. In the third period, between September 1998 and November 1999, we performed routinely serum β-HCG determination in almost every case and, if serum β-HCG determination the day before aspiration was negative, or was positive but in low concentrations (arbitrarily <80 IU/ml), a fresh dose of HCG was given, rescheduling aspiration 34 or 36 h later. This situation was observed in four cases, avoiding the presence of EFS.

Results: In the first period, in the first five cases, all follicular fluids aspirated lacked granulosa cells or they were immature. A total of five oocytes were recovered, all of which were at prophase I stage. In the other four patients of the first period, 32 oocytes were recovered: 25 (78%) were at metaphase II stage. In all cycles intracytoplasmic sperm injection (ICSI) was performed, 60% of the oocytes injected fertilized with normal cleavaged embryos. No pregnancy was achieved. In the second period, normal cleavaged embryos were obtained, and one single pregnancy was achieved. Two pregnancies were achieved in the third period.

Conclusions: We consider performing routinely in all of our patients a serum dosage of β-HCG 16 h after HCG administration, although the minimum concentration of β-HCG necessary to obtain mature oocytes post-follicular aspiration it is not clear. Once EFS diagnosis is made, aspiration should be stopped in order to leave as many follicles as possible to be aspirated after a new dose of HCG. If, 16 h after HCG administration we have a negative or doubtful urinary test or a serum HCG determination negative or in low concentrations (perhaps <80 UI), we have to reschedule the retrieval after a second HCG administration. New studies are needed to establish the level of β-HCG, 16 h post 10 000 UI administration, below which we have to cancel and reschedule oocyte retrieval.

P-138. Ultrasound-guided embryo transfer improves pregnancy rates

Kolankaya A., Enginsu M.E., Çamlıbel T.F., Sarıkamış B., Kurt E., Yıldız K. and Hünerli Ç.

Metropolitan Florence Nightingale Hospital, Assisted Reproductive Technologies Department, Gayrettepe Mahallesi Cemil Aslan Güder sokak No:8 Beşiktaş-İstanbul, Turkey

Introduction: The role of abdominal ultrasonography during embryo transfer is still open to debate. This study evaluates the direct effect of ultrasound (US)-guided embryo transfer on pregnancy rates by correcting the number and the quality of the embryos transferred.

Materials and methods: A total of 359 embryo transfer procedures following intracytoplasmic sperm injection (ICSI)/embryo transfer cycles were evaluated in a retrospective manner. In 252 of the transfers, US guidance was not used (clinical touch embryo transfer); whereas in 107 a careful US examination was carried out (US-guided embryo transfer). In all 359 transfers two or more embryos were transferred and the transferred embryo scores were >74% (referring to embryos that were either grade 1 or grade 2). All transfers were carried out on either the 2nd or the 3rd day after the ICSI procedure. On the 12th day following embryo transfer, a serum level of >15 mIU/ml β-HCG was considered as a positive pregnancy, and completion of the first trimester was considered as an ongoing pregnancy. Transfers were carried out with either Wallace–Edwards or Wallace malleable catheters. The data obtained were compared using χ^2 analysis and Students *t*-test.

Results: There was no significant difference between the two groups in respect to age, number of oocytes retrieved, and number of embryos transferred. The only differences between clinical touch embryo transfer group and US-guided embryo transfer group were in the pregnancy (52.4 versus 69.2%) and ongoing pregnancy rates (40.5 versus 52.3%) respectively ($P < 0.05$) (Table 1).

Table 1. Comparison of different ET protocols on the basis of the assisted reproduction technology (ART) outcome

	Clinical touch ET	US-guided ET
Patients (*n*)	252	107
Age (AVR±SD)	30.9 ± 4.8	30.9 ± 4.9
Oocytes collected (AVR±SD)	12.7 ± 5.1	12.8 ± 4.8
Embryos transferred (AVR±SD)	4.0 ± 0.59	3.98 ± 0.51
Positive pregnancy (%)	132 (52.4)[a]	74 (69.1)[b]
Ongoing pregnancy (%)	102 (40.5)[c]	56 (52.3)[d]

$P < 0.01$ a versus b; $P < 0.05$ c versus d.

Discussion: The fate of the implantation stage is dependent on several factors, among which the first and probably one of the most important is the embryo transfer procedure. Unfortunately there is scarce data in the literature as to the benefits or otherwise of US-guided embryo transfer. The results of this study demonstrate that in patients who have a fair chance of pregnancy, US-guided embryo transfer has a beneficial effect. This may be due partly to a full bladder affecting the position of the uterus during the procedure. In addition, the catheter remains visible throughout the procedure, and the clinician does not have to touch the uterine fundus. This study therefore suggests that US-guided embryo transfer enhances the pregnancy rates in ART.

Contraception

P-139. Oral contraceptives with 20 mg ethinyloestradiol reduce serum levels of vitamin B_{12}

Rieger L., Sütterlin M., Bussen S., Dietl J. and Steck T.

Department of Obstetrics and Gynecology, Ludwig-Maximilians-University of Würzburg, Josef-Schneider-Str. 4, D-97080 Würzburg, Germany

Introduction: Reports on the effect of oral contraceptives (OC) on the serum levels on vitamin B_{12} (cobalamin) and folic acid are contradictory. We examined the impact of preparations with 20 mg ethinyloestradiol (EE) on the metabolism of these vitamins.

Materials and methods: We determined the folate and cobalamin status in 71 nulligravidae women taking OC of the combination type with 20 mg EE (study group) for at least 3 months (mean 30.1 ± 28.2 months). If the contraceptive preparation had been changed within the last months, only those women taking the same pill with 20 mg EE for at least 1 year were included in the study. The mean age was 24.4 ± 4.5 years (range 18–38). The mean body mass index (BMI) was 21.4 ± 2.4 kg/m^2 (range 17.0–28.1). The control population consisted of 170 non-selected nulligravid women of similar reproductive age [mean ± SD 25.4 ± 4.8 years, range 16–40, $P = 0.11$ (*t*-test)] and body mass index (21.8 ± 2.7 kg/m^2, range 16.8–29.3). None of the subjects of both collectives had a known endocrine dysfunction or suffered from gastrointestinal, renal, or neurological disease. Women receiving vitamin supplementation or any other medication were excluded. After fasting venous blood sampling in the pre-ovulatory phase of the menstrual cycle, serum levels of folate and vitamin B_{12} were measured by an automated microparticle–enzyme–intrinsic-factor assay (AxSYM B12, Abbott, Wiesbaden, Germany) and an ion-capture-assay (AxSYM Folsäure, Abbott) according to the instructions of the manufacturer. The normal range of concentrations for folic acid was 3.1–12.4 ng/ml and for vitamin B_{12} was 223–1132 pg/ml.

Results: Women taking OC with 20 mg EE had a significantly [$P < 0.001$ (*t*-test)] lower mean serum level of cobalamin (328 ± 138 pg/ml, range 124–761 pg/ml) compared to controls (558 ± 248 pg/ml, range 190–1665 pg/ml). The percentages of reduced, normal, and elevated vitamin B_{12} values were significantly different in the study and control group too [$P < 0.001$(χ^2-test)]. There was no significant difference [$P = 0.72$ (*t*-test)] between the mean folate values in the study (9.9 ± 2.7 ng/ml, range 3.8–15.5 ng/ml) and control group (9.8 ± 3.8 ng/ml, range 3.1–24.2 ng/ml). Furthermore, the rate of reduced or elevated concentrations of folic acid was similar [$P = 0.76$ (χ^2-test)] in both collectives. The majority (70%) of women (56% of the study and 75% of the control group) had both vitamin concentrations within the normal range. However, 28 women had low cobalamin values but had not reported symptoms. None of the subjects in the study or control group suffered from folate deficiency. Neither age, body mass index, vegetarian diet, nor smoking had a significant influence on the vitamin levels in any of the collectives. A significant association of both substances was not observed in the study group [$P = 0.38$ (ρ)], but in the control collective ($P = 0.001$) and in the entire population ($P = 0.015$). In the study group, there was no association between the duration of oral contraception or the various contraceptive preparations and vitamin concentrations.

Conclusion: Oral contraceptives of the combination type containing 20 mg ethinyloestradiol reduce the serum level of vitamin B_{12} significantly, without having a substantial clinical effect. The levels of folic acid are not altered by such contraceptives; in particular, there is no association of folate deficiency and this medication.

Early Pregnancy

P-140. Characterization of HLA-G, HLA-G receptor expression, and of maternal leukocytes in tubal versus uterine placentation

Pröll J.[3], Goffin F.[1,2], Bensussan A.[3], Berrebi A.[3], Frankenne F.[1], Foidart J.M.[1,2] and Le Bouteiller P.[3]

[1]Departments of Tumor and Development Biology, CHU, [2]Obstetrics and Gynecology, University of Liège, Belgium, and [3]Inserm U395, CHU Purpan, Toulouse, France

Background: Human placentation is characterized by the onset of a haemochorial circulation, allowing fetomaternal exchange. The high blood flow in the intervillous chamber required for fetal development is the consequence of maternal vascular remodelling by a subset of extravillous cytotrophoblastic cells (EVCT), which penetrate deeply into the maternal decidua and invade the maternal uteroplacental arteries. At the fetomaternal interface, the decidua is massively infiltrated by natural killer (NK) cells that enter in close contact with the invading EVCT. In the uterus, the NK cells are believed to control the cytotrophoblast differentiation and invasion by recognizing the non-classical HLA-G antigen expressed on the EVCT surface through specific receptors. This immunological recognition constitutes the basis of the maternal immunotolerance, avoiding the rejection of the fetal allograft.

Objective: By comparing normal uterine pregnancies (UP) and gestational age matched ectopic tubal pregnancies (TP), we addressed the following questions: (i) Is HLA-G expression by EVCT dependent on maternal tissue environment? (ii) Are maternal leukocyte populations specific to the implantation site? (iii) Are HLA-G receptors expressed on tubal leukocytes?

Materials and methods: Viable tubal and uterine pregnancies collected during surgical procedures were frozen in liquid nitrogen or fixed in formalin. Immunohistochemical analyses were performed to assess the HLA-G and HLA-G receptor expression and to characterize the leukocyte population at the implantation site.

Results: HLA-G expression by all subsets of EVCT was identical in both UP and TP. Maternal leukocyte populations differed considerably when comparing tubal versus uterine implantation sites. In TP, NK cells (CD56+) were absent and macrophages (CD14+) were significantly less often detected, whereas T cells (CD8+) and dendritic cells (CD1a+) were much more represented. The HLA-G receptors (LIR1/ILT2, LIR2/ILT4, BY55) were also differentially expressed, with LIR1/ILT2, LIR2/ILT4 and BY55 being more frequently detected in TP than in the decidua