

OHSS on the DET. Pts with  $\geq 12$  oocytes and/or oestradiol  $\geq 2500$  pg/ml were defined as high-risk-patients and separately randomized to receive either protocols II or III. These groups were defined as groups IV and V respectively. Complete data of 241 pts were available at the time, when this abstract was written.

**Results:** 50, 42, 42, 48, and 59 pts were randomized to protocol I, II, III, IV, and V, respectively. Pregnancy rates (PR) were 26.0, 19.0, 21.4, 27.1 and 28.8% in the five protocols. Overall PR was 24.9%, PRs in the low-risk (I-III) and high-risk groups (IV,V) were 22.4 and 28.0% respectively. Implantation rates (IR) were 10.6, 11.9, 8.8, 13.7 and 11.3% respectively. There was no significant difference between the groups regarding PR or IR. Mean age was  $31.84 \pm 3.3$ ,  $34.04 \pm 3.8$ ,  $33.52 \pm 4.2$ ,  $31.02 \pm 3.3$ , and  $31.39 \pm 4.0$  years, respectively. There was a significant difference (Kruskal-Wallis test, KWT) between group II versus groups IV and V, as well as between groups III versus IV ( $P < 0.05$ ). Body mass index was not different between the groups. Mean oestradiol was significantly higher in groups IV and V, compared to groups I, II, and III ( $P < 0.05$ ). The same held for the number of oocytes retrieved ( $7.40 \pm 2.6$ ,  $6.71 \pm 3.2$ ,  $7.55 \pm 2.9$ ,  $14.78 \pm 5.2$ ,  $14.59 \pm 5.3$ , in groups I, II, III, IV, and V respectively). The cumulative embryo score was significantly higher in group V ( $29.95 \pm 8.3$ ), compared to group II ( $22.31 \pm 11.7$ ) and III ( $23.33 \pm 10.5$ ) (KWT,  $P < 0.05$ ). Two severe OHSS were observed, one each in groups IV and V. Three moderate OHSS occurred in the low-risk group, all in group I, and one occurred in group V. Nineteen mild OHSS were recorded, two in each of the low-risk groups, six in group IV and seven in group V.

**Conclusion:** There is no indication to use HCG in LPS, since there is no significant difference in PR between the groups. There is a higher risk of OHSS after HCG administration. The slight, non-significant differences observed between the PR are mainly due to the differences in age and embryo quality, which occurred despite a randomization system.

#### 10.30-10.45

##### O-005. Intracytoplasmic sperm injection versus in-vitro fertilization in pure tubal factor infertility: a prospective randomized trial

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**Introduction:** It is highly controversial whether intracytoplasmic sperm injection (ICSI) will replace standard in-vitro fertilization (IVF) in patients with pure tubal factor infertility. The aim of this prospective randomized study is to compare ICSI versus IVF in women with tubo-peritoneal factor infertility.

**Materials and methods:** Patients with tubo-peritoneal factor infertility were randomized to ICSI ( $n = 38$ ) or IVF ( $n = 38$ ). Semen analysis including morphology assessment according

to strict criteria were normal in all couples. All patients had luteal-long gonadotrophin-releasing hormone analogue protocol and standard procedures for ICSI and IVF. Chi-square and independent *t*-tests were used for statistical analyses.

**Results:** These are given in Table I.

**Table I.** Baseline characteristics and ICSI/IVF results.

	ICSI-ET	IVF-ET
Embryo transfer cycles ( <i>n</i> )	38	38
Female age (years)	$34 \pm 3.96$	$34.03 \pm 3.77$
Male age (years)	$37.46 \pm 4.15$	$37.34 \pm 4.75$
Duration of infertility (years)	$10.08 \pm 4.19$	$9.06 \pm 4.59$
Patients with uni/bilateral hydrosalpinx ( <i>n</i> )	4.0	4.0
Oestradiol level-HCG (pmol/l)	$5193.7 \pm 3764$	$5071 \pm 3060$
Oocytes ( <i>n</i> )	$9.08 \pm 6.22$	$8.11 \pm 3.7$
Metaphase-II oocytes ( <i>n</i> )	$7.32 \pm 4.34$	$7.0 \pm 3.5$
2-pronuclei rate (%)	$69.03 \pm 22.12$	$67.32 \pm 25.01$
Cleavage rate (%)	$66.87 \pm 23.04$	$69.84 \pm 23.73$
Embryos ( <i>n</i> )	$4.97 \pm 3.06$	$5.0 \pm 3.17$
Grade-I embryos ( <i>n</i> )	$3.45 \pm 2.43$	$3.13 \pm 2.54$
Embryos transferred ( <i>n</i> )	$3.39 \pm 1.39$	$3.61 \pm 1.59$
Grade-I embryos transferred ( <i>n</i> )	$2.71 \pm 1.71$	$2.5 \pm 1.43$
Individual implantation rate (%)	$38.75 \pm 24.46$	$34.58 \pm 16.97$
Clinical pregnancy/ET (%)	21.05	21.05
Take-home baby/ET (%)	18.42	15.79

ET = embryo transfer

All results are non-significant.

**Conclusion:** ICSI outcome and pregnancy results are comparable to those of IVF in patients with pure tubo-peritoneal factor infertility.

#### 10.45-11.00

##### O-006. Computerized fast-motion video capture of mouse embryo development and hatching: a comparative analysis between normal and assisted hatching

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**Introduction:** Embryo development and hatching are difficult to observe as they take place inside an incubator and last several days. Our goal was to develop a computerized video capture system which allows the observation, recording and analysis of these phenomena using fast-motion movies, while keeping the embryo in an environment (temperature, light, CO<sub>2</sub>, medium) suitable for its growth. Our first recordings were aimed at comparing the development of control and zona pellucida (ZP)-drilled mouse embryos.

**Materials and methods:** Zygotes are collected from female mice and cultured for 1 day in carbonated human tubal fluid medium supplemented with 5 mg/ml bovine serum albumin. ZP microdrilling is performed with a single 30 ms shot using a 1.48  $\mu$ m diode laser system (Fertilase; Medical Technologies Montreux, Switzerland), according to a technique described elsewhere [Germond M. *et al.* (1995) *Fertil. Steril.*, **64**, 604-

611]. A single 2-cell embryo is transferred into a 10 µl culture medium droplet under mineral oil inside a Petri dish, fitting into an in-house constructed humidified micro-incubator, maintained at 37°C by water circulation and gassed with 5% CO<sub>2</sub>. A CCD video camera mounted on the microscope sends a signal to a frame grabber card located in a computer. The image is digitized with a time lapse of several minutes and stored as a file with a codified name generated automatically by the computer software. Microscope light source and image acquisition frequency are controlled by visual basic software (PA Images, Switzerland), which also generates the movies from the stored image sequences.

**Results:** The specially designed micro-incubator offers an environment compatible with embryo development. Normal cleavage rates and hatching occur over the 4–5 days of culture with only a need for medium renewal. In control embryos, several extension/compaction movements (ECM) led to a progressive thinning and to a sudden large slitting of the ZP through which the expanded blastocyst quickly escapes. The laser-drilled embryos start hatching 1 day earlier than the controls by protruding through the drilled hole. The ECM do not lead to ZP thinning and rupture; more ECM are needed before hatching is completed.

**Conclusion:** We hereby present a new research tool which allows us to study the hatching phenomenon as well as all the other stages of the in-vitro development in embryos of all species. The computerized images provide easily accessible material for different measurements. The movies can be made over longer periods of time than normal videos as they are compiled to accelerate phenomena usually difficult to observe.

#### 11.00–11.15

##### **O-007. Maturation in-vitro of mouse prenatal follicles recovered from ovarian grafts under the kidney capsule**

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**Introduction:** Thousands of primordial follicles are present in the ovaries of neonatal mammals. Until now, reproducible conditions for a complete development of primordial follicles *in vitro* have not been established. The aim of this study was to achieve complete development of primordial follicles in newborn mouse ovaries by combining in-vitro and in-vivo steps. A two-step strategy was set-up. First the ovaries from newborn mice were transplanted heterotopically under the kidney capsule of ovariectomized adult female recipients; after 14 days, preantral follicles were isolated mechanically from the recovered grafts and cultured *in vitro* for 12 days.

**Materials and methods:** Six- to eight-week-old F1 (C57BL/6j×CBA/Ca) female ovariectomized mice were used as recipients. Newborn F1 female mice ( $n = 6$ ) were killed and the ovaries were dissected free. Five ovaries that were not transplanted were placed into Bouin's fluid and processed for

histological examination. One newborn mouse ovary was transplanted under the renal capsule of each recipient ( $n = 7$ ). Two weeks after transplantation, preantral follicles were isolated from four ovarian grafts and three recovered graft ovaries were prepared for histological examination. The preantral follicles were cultured individually in 20 µl α-MEM medium supplemented with 100 mIU/ml recombinant follicular stimulating hormone (rFSH) and 5% FCS in 60 mm petri dishes incubated at a condition of 37°C, 100% humidity, and 5% CO<sub>2</sub> in air for 12 days. Half of the medium was refreshed every 2 days. At day 12 of culture, final oocyte maturation was induced by adding 2.5 IU/ml human chorionic gonadotrophin (HCG). The nuclear maturation of oocytes was assessed after denudation from granulosa cells. Preantral follicles ( $n = 97$ ) isolated from 14-day-old mice were cultured under the same conditions as control group.  $\chi^2$  test was used for statistical analysis.

**Results:** All grafts ( $n = 7$ ) were recovered 2 weeks after transplantation. Histological examination of recovered grafts ( $n = 3$ ) revealed that follicles proceeded to preantral and antral follicle stages with a predominance of preantral follicles. Approximately 15–25 preantral follicles were isolated mechanically from each ovarian graft ( $n = 4$ ) and total 64 selected preantral follicles (100–150 µm in diameter) were put into culture. There were differences between the control and the transplanted groups in the percentage of follicle survival during 12 days in-vitro culture (98 versus 64% respectively;  $P < 0.0001$ ), and in the percentage of oocytes that acquired competence to undergo germinal vesicle breakdown (GVB) (91 versus 65% respectively;  $P < 0.0001$ ) or in the percentage of GVB oocytes that produced a polar body, indicating progression of meiosis to metaphase II (86 versus 46% respectively;  $P < 0.001$ ).

**Conclusion:** The results reported here show that maturation of mouse primordial follicles is possible by in-vivo transplantation combined with in-vitro culture. This two-step method may be a potentially attractive alternative to promote the transition from primordial to preantral follicles that can be supported by an in-vitro culture system.

#### 11.15–11.30

##### **O-008. Impact of stages III–IV of endometriosis on the outcome in recipients of oocytes from the same donor: matched case-control study**

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**Introduction:** Infertility in endometriosis patients has been attributed to alterations within the oocyte which, in turn, result in embryos of lower quality and reduced ability to implant. The aim of this study was to compare the in-vitro fertilization and embryo transfer (IVF/ET) outcome in oocyte recipients with and without severe endometriosis receiving eggs from the same donor.