the day of embryo transfer at in-vitro fertilization (IVF) treatment was correlated with hormonal profiles and the receptivity of the uterus.

Materials and methods: The subjects are 149 patients who underwent IVF–embryo transfer treatment at our hospital from March 1997 to December 1998. Transvaginal ultrasonography with colour Doppler imaging and pulsed Doppler spectral analysis was performed just before embryo transfer. The thickness of the endometrium and the pulsatility index (PI) of the ascending uterine arteries (right and left, separately) were measured. Immunoassays were used to measure the serum concentrations of oestrogen and progesterone in the early morning on the day of embryo transfer.

Results: (i) Serum oestrogen values of <1200 pg/ml were negatively correlated with the PI value ($P < 0.005$). When oestrogen was >1200 pg/ml, the PI value tended to be high; (ii) serum values of progesterone were negatively correlated with the PI value ($P < 0.001$); (iii) the oestrogen/progesterone ratio, the thickness of the endometrium, and the patient's age were not correlated with the PI value; (iv) patients ($n = 11$) who had past history of unilateral ovary operations had 37.6% higher PI values for the operated than the intact side. In contrast, 51 patients who had not undergone operations demonstrated a 20.7% difference between the left and right PI values, this being significant ($P < 0.05$); (v) there were 18 patients for whom treatment was successful with live births or on going pregnancies, and 37 who had received at least one high-grade embryo but had failed to become pregnant. The PI value was significantly lower in the successful group (2.120 ± 0.363) than in the failure group (2.652 ± 0.835) ($P < 0.005$). Other factors were; serum oestrogen value of 438.6 ± 438.0 pg/ml versus 328.6 ± 239.7 pg/ml, serum progesterone value of 55.25 ± 33.38 ng/ml versus 52.61 ± 21.76 ng/ml, an oestrogen/progesterone ratio of 7.64 ± 3.44 versus 5.991 ± 2.47, thickness of endometrium of 12.6 ± 3.1 mm versus 11.9 ± 2.7 mm, and patient's age of 34.3 ± 3.5 years versus 35.3 ± 2.7 years. None of these exhibited any significant difference.

Conclusion: The PI value for ascending uterine arteries measured by transvaginal colour Doppler ultrasonography on the day of embryo transfer during IVF treatment was correlated with the serum values of oestrogen and progesterone, and could be helpful in predicting the receptivity of the uterus.

P-119. Fertilization rates in sibling oocytes from endometriosis patients undergoing IVF and ICSI

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Introduction: Endometriosis is an enigmatic disease related to infertility. There is a suspicion that endometriosis may cause impaired oocyte quality and fertilization rate, which could partially explain the associated subfertility. The aim of this study was to compare the fertilization rates achieved through conventional in-vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) in sibling oocytes of patients with endometriosis, with no male factor associated.

Methods: In 23 IVF cycles (from 23 patients) due to endometriosis, mature oocytes (metaphase II) of each patient were divided in two groups: half were fertilized using IVF ($n = 150$) and the other half were fertilized using ICSI ($n = 132$). Ovarian stimulation was carried out with leuprolide acetate and human menopausal gonadotrophin (HMG). Oocyte retrieval was carried out 35 h after administration of human chorionic gonadotrophin (HCG), by vaginal ultrasound, and semen was prepared by Percoll gradient. For IVF, 100 000 motile spermatozoa per oocyte were placed in a drop of 100 µl under oil. Human tubal fluid (Irvine Scientific) was used as the culture medium. ICSI was carried out as described by Van Steirteghem. The eggs were observed 18 h after insemination and were considered to have been fertilized if two pronuclei and two polar bodies were present.

Results: See Table I. The fertilization rate in these IVF cases was lower than the general fertilization rate of our lab (52%; $P < 0.01$) including male factor.

Table I.

	Inseminated oocytes (*n*)	Fertilized oocytes (*n*)	Fertilization rate (%)
ICSI	132	106	80
IVF	150	57	38*

ICSI = intracytoplasmic sperm injection; IVF = in-vitro fertilization.
*$P < 0.001$.

Conclusion: Lower fertilization rates might be a possible cause of infertility in patients with endometriosis. The actual cause or mechanisms involved in the lower fertilization rates for patients with endometriosis is still unclear, warranting further studies.

P-120. Sperm activation assay with a *Bufo arenarum* toad egg extract in the evaluation of sperm fertilizing ability after ICSI

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Introduction: Conventional techniques used to evaluate the fertilizing ability of spermatozoa in standard in-vitro fertilization (IVF) do not have the capability to predict the capacity of achieving successful fertilization rates after intracytoplasmic sperm injection (ICSI). We have developed the human sperm activation assay with a *Bufo arenarum* egg extract that promotes sperm nuclear decondensation, the first visible change that occurs during sperm activation, and subsequent pronuclear formation. Diagnostic utility of the assay was tested, correlating the sperm activation rate with the results of fertilization obtained in a group of patients undergoing an ICSI procedure.

Materials and methods: Adult *B.arenarum* toad eggs were obtained through ovulatory discharge produced 12 h after injection of 2500 IU human chorionic gonadotrophin (HCG).

In order to obtain the extract mature eggs were lysed by centrifugation at 10 000 *g* for 15 min. Two techniques for sperm permeabilization were tested, in one Triton X-100 was used, the other was using lysolecithin, in both cases 50 mM dithiothreitol (DTT) was added. Treated spermatozoa were then mixed with egg extract and incubated at 37°C, for 10 min. The reaction was halted with formaldehyde and smears were prepared and then stained with Giemsa, counting the percentage of decondensed sperm heads observed. Sperm activation assay was performed on 62 semen samples from couples undergoing ICSI cycles in our assisted reproductive technology (ART) programme due to severe male factor.

Results: The highest sperm head decondensation was observed after a 10 min incubation period. No differences were observed when comparing the use of fresh versus cryopreserved semen samples in the assay. Predictive value of the sperm activation assay was determined after analyzing activation of 62 semen samples. A cut-off value of 88% for sperm activation was observed, where normal fertilization rates after ICSI were ≥50% in 95% of the patients. Therefore a percentage ≥88% was established as a positive predictive value whereas ≤59.5% was considered as negative one.

Conclusions: *B.arenarum* egg extract is capable to promote decondensation and recondensation of human spermatozoa with high efficiency. The methodology employed is highly reproducible and representative of the sample. The main parameters for a sperm activation assay were established. Fresh as well as cryopreserved semen samples may be used. Sperm activation assay with *B.arenarum* egg extract could be a useful diagnostic tool to predict successful fertilization by ICSI. The potential usefulness of this assay to evaluate the fertilizing ability in prospective ICSI/IVF patients has to be established.

P-121. Frozen–thawed embryo transfer. Do we thaw embryos the day of transfer or the day before?

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Introduction: Selection of best morphology thawed embryos, is one of the most important factors predicting implantation. Following this reasoning, the objective of this study was to go one more step, selecting those thawed embryos that were furthest advanced in terms of cleavage 24 h post-thawing.

Materials and methods: This is a prospective, randomized study of 110 successive thawing cycles, in which embryos were transferred the same day of thawing procedure (n = 54) or 24 h later (n = 56). All embryos had been frozen at their first cleavage stage. In the first group embryos with ≥50% of intact blastomeres after thawing, were transferred. In the second group, cleaved embryos were transferred after 24 h post-thawing culture. Statistical analysis was performed using χ^2 test.

Results: Main results are shown in Table I.

Table I.

	Group 1	Group 2
No. of thawing cycles	54	56
No. of thawed embryos	238	270
No. of embryos with ≥50% of intact blastomeres (%)	135 (56.7)	161 (59.6)
No. of cleaved embryos (%)	–	90 (55.9)
No. of transfers	43	36
No. of embryos transferred	135	90
No. of pregnancies (%)	8 (18.6)	7 (19.4)

Conclusion: There is no difference in terms of pregnancy rate in both groups. These results suggest there is no detrimental effect in further in-vitro cultured of thawed embryos and this approach could be useful for laboratory organization.

P-122. Zona dissolution enhances pregnancy for ICSI patients

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Introduction: We have observed that intracytoplasmic sperm injection (ICSI) gives lower blastocyst formation rates than in-vitro fertilization (IVF) [Wun, W.S.A., Dunn, R.C., Valdes, C.T. *et al.* (1997) *Chinese J. Physiol.*, **40**, 237–242]. Polyvinyl-pyrrolidone (PVP) is not the responsible factor for the low blastocyst formation. Our data suggests mechanical trauma is the responsible factor. Zona dissolution was reported to enhance pregnancy for difficult cases [Fong, C.Y., Bongso, A., Ng, S.-C. *et al.* (1997) *Hum. Reprod.*, **12**, 557–560]. This study is to examine whether zona dissolution can enhance pregnancy for ICSI patients or not.

Materials and methods: All cases of assisted reproductive technology (ART) between June 1, 1996 and December 31, 1997 were included in the study. All cases were transferred on day 5 at blastocyst stage. ICSI was carried out and the embryo sac was examined. χ^2 tests were used for each age category. The overall statistical analysis was Multiple Logistic Regression with control of age.

Results: See Table I.

Table I.

	Age (years)	Cases (n)	No. (%) pregnant	No. of embryo transfers	Implantation (n)
Control	Age ≤34	79	26 (32.9)[a]	257	35 (13.6)[b]
	Age 35–39	66	14 (21.1)	245	17 (6.9)
	Age ≥40	22	9 (40.9)	81	12 (14.8)
	Total	167	49 (29.3)	583	64 (10.9)
ZD	Age ≤34	17	12 (70.5)	65	17 (26.1)
	Age 35–39	18	7 (38.8)	68	8 (11.7)
	Age ≥40	15	3 (20)	49	4 (8.1)
	Total	50	22 (44)	182	29 (15.9)
Control versus ZD	Age ≤34		$P < 0.05$		$P < 0.05$
	Age 35–39		NS		NS
	Age ≥40		NS		NS
	Total		$P < 0.05$		NS

[a]Pregnancy percentage; [b]Implantation percentage.
ZD = zona dissolution; NS = not significant.