

Results of microinjection are shown in Table 3. After in-vitro culture (IVC), the most advanced embryonic stage obtained was 150 – 200 cell embryos (2 morulae).

Table 2. Number of recovered COCs

Follicle diameter	<20 mm	20–30 mm	≥30 mm
cc	35	13	1
cr	6	8	2
exp	2	2	4

Table 3. Results of microinjection (number of oocytes)

Oocytes	cc	cr	exp	Total
In-vitro matured	49	16	7	72
Microinjected (%)	26	9	6	41 (57)
Successful ICSI (%)	20	6	5	31 (75.6)
Fertilized ova	19	5	5	30
2–8 cells (%)	8	2	2	17 (54.8)
9–16 cells	1	0	0	1
> 16 cells	4	0	0	4

The cleavage rate obtained in this study is similar to the one obtained in previous experiments with oocytes from slaughtered mares (55%, $n = 41$ versus 46%, $n = 103$) [Guignot *et al.*, (1998) *Reprod. Nutri. Dev.*, **38**, 653–663]. However, microinjection of oocytes collected in this study has enabled a more advanced embryonic development after IVC (embryos of 150–200 cells after 8 days versus embryos of 50 and 80 cells after 7 days).

In conclusion, a weekly ultrasound-guided oocyte aspiration from cyclic mares without stimulating treatment enabled a collection of 2.1 oocytes/mare/week. The mare fertility per cycle in terms of rate of cleaved ova after ICSI is about 1.5 (0.5 cleaved ova/mare/week), while it is 0.6 in terms of collected embryo after in-vivo fertilization. The next step will be to transfer to recipient mares the embryos obtained after ICSI and IVC.

P-079. Plasma leptin values after gonadotrophin stimulation

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Introduction: Leptin (LEP), a product of ob gene expression, is a proteic hormone composed of a single 16-kDa chain. It is synthesized almost exclusively in adipose tissue and acts upon the CNS, particularly the hypothalamus, suppressing the ingestion of food and stimulating energy consumption. In addition to these metabolic effects, there is evidence that LEP acts upon other endocrine axes, and results from experimental and clinical studies have suggested its participation in mammalian fertility.

Material and methods: Normal subjects were 16 women, 20–49 years old, with no apparent pathology. Study group com-

prised 14 patients, 29–39 years old, under ovarian stimulation treatment for in-vitro fertilization. LEP and oestradiol determinations were carried out at the time of suppression control with GnRH agonists and, later during the stimulation phase, with FSH. Oestradiol was determined with an automated fluorometric method (Vidas Merieux France), and LEP with an ELISA technique (DRG Germany).

Results: In the suppression phase, LEP values did not differ from those obtained in the normal, untreated reference group (11.9 ± 5.6 ng/ml) versus (11.8 ± 6 ng/ml) ($P > 0.05$). After 8–10 days of treatment with FSH stimulation, values reached 19.5 ± 8.6 ng/ml ($P < 0.05$), implying a 91% increase over the suppression values. Later, LEP values decreased with respect to the peak value. In eight patients (group A), the decrease as compared to the peak was –23.2%, whereas in the other six patients (group B) there was no change or a substantially smaller decrease (–1.7%) ($P < 0.05$).

Discussion: At present we have no explanation for this phenomenon, which contrasts with the progressive rise of oestradiol over the extent of the treatment. Our study shows that there is a strong association between pregnancy and LEP decreases (group A) ($P = 0.01$); i.e. all the women in group A and none of the women in group B became pregnant. We speculate that a descent of LEP values may indicate the culmination of oocyte maturity and could be used in clinical practice as a sign of favourable prognosis in patients undergoing in-vitro fertilization.

P-080. Oocyte donation programme: influence of age and aetiology and improvement of uterine blood flow velocity and pregnancy outcome after low-dose aspirin treatment

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Introduction: Uterine age has been postulated to influence the process of embryo implantation. In previous studies we have demonstrated that low-dose aspirin treatment increases implantation rate (IR) and pregnancy rate (PR) and improves blood flow velocity (BFV) in patients undergoing ART procedures (Suppl., *Fertil. Steril.*, 1998; O-320). The objective of this study was to evaluate the influence of age and ovarian failure aetiology and uterine BFV on IR and PR in recipients treated with aspirin in a prospective, randomized, double-blind, placebo study.

Materials and methods: Seventy-nine oocyte donation cycles due to different aetiologies were included in our oocyte donation programme. They were randomly subdivided into two groups: group A ($n = 41$), mean age 38.4 years (26–53) received 100 mg/day aspirin, and group B ($n = 38$), mean age 38.0 years (26–50) received placebo. Groups A and B were subdivided according to the recipient age. A1 and B1, <40 years; A2 and B2, 40–45 years; and A3 and B3, >45 years. Oocyte donors were patients undergoing ART cycles who wished to donate their excess oocytes anonymously and altruistically. Thirty-three of the 79 recipients were cycling

and the remaining 53 were non-cycling women. Cycling recipients underwent pituitary down-regulation using GnRH analogue from the midluteal phase of the previous cycle. All patients received hormonal replacement therapy (HRT) with increasing oral doses of micronized 17 β -oestradiol starting on day 2 of the cycle. The luteal phase was supported with oily progesterone (100 mg/day/i.m.) and vaginal micronized progesterone (400 mg/day). Recipients' uterine BFV [expressed as pulsatility index (PI)] was assessed by transvaginal colour Doppler ultrasound on day 2 of an HRT previous cycle and on the day of progesterone commencement. Data was analysed using Student's *t*-test and Fisher's exact test.

Results: PI on day 2 of a previous cycle and on day of progesterone commencement was 2.12 ± 0.42 versus 1.19 ± 0.31 respectively for group A ($P < 0.05$) and 2.01 ± 0.45 versus 1.98 ± 0.42 respectively for group B (NS). On day 2 of the previous cycle PI was similar in both groups but on day of progesterone commencement PI was significantly lower in the aspirin-treated group as compared to placebo group ($P < 0.05$). PI was 1.26 and 2.3 for pregnant and non-pregnant recipients respectively ($P < 0.05$). No patient with a PI over 3.5 achieved pregnancy. PI in cycling patients was 1.89 ± 0.38 versus 1.93 ± 0.39 in non-cycling patients. Clinical PR was 68.29 versus 31.58% ($P < 0.05$) and IR was 25.90 versus 14.41% ($P < 0.05$) for group A and B respectively. PI of subgroups A1, A2, and A3 were compared to B1, B2, and B3 and presented no significant differences. IR and PR were not significantly different between groups A1, A2, and A3 and between groups B1, B2, and B3. PI, IR, and PR were similar in all groups of patients independently of the aetiology.

Conclusions: Age and ovarian failure aetiology did not influence PI, IR, and PR. Our results suggest that pregnancy outcome is not affected by uterine age. Pregnant patients showed significantly lower PI values than non-pregnant patients. Low-dose aspirin treatment has demonstrated effectiveness in improving uterine blood flow velocity, implantation rate, and pregnancy rate in patients undergoing oocyte donation cycles.

P-081. Blastocyst transfer is associated with an increased incidence of monozygotic twinning

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Introduction: Increased implantation and pregnancy rates following blastocyst transfer (BET) using chemically defined media have now been reported by several investigators. We have also reported an increased incidence of multiple pregnancy following BET. Dizygotic twinning is an established risk associated with assisted reproductive technology (ART) and monozygotic twinning has also been reported, especially in relation to assisted hatching. Since BET is a relatively novel

treatment modality for most centres in the USA, no one programme has sufficient volume to draw adequate conclusions from their experience. In this report, we present data from multiple centres, examining the rate of monozygotic twinning associated with BET using commercially available, cell-free culture systems with unmanipulated blastocysts.

Materials and methods: IVF patients were stimulated using standard GnRH α /FSH protocols after signing approved consent forms. Oocytes were retrieved transvaginally under ultrasound guidance and were inseminated conventionally or with ICSI. Embryos were either cultured in P1 supplemented with 10% synthetic serum substitute (SSS) from day 0–3 and then were transferred into blastocyst medium supplemented with 10% SSS for an additional 48 h, or were cultured in IVF50 from day 0–3 and then were transferred into S2 for an additional 48 h. Blastocysts exhibiting a well-defined trophoctoderm, a distinct inner cell mass, and an expanded blastocoele cavity were transferred to the uterus on day 5. Assisted hatching was not used. Monozygotic twin pregnancy was defined as two fetuses seen within the same gestational sac identified on ultrasound examination.

Results: A total of 199 BET pregnancies were examined at multiple centres throughout the country. A monozygotic twin gestation occurred in 10 of these pregnancies (5%). All were monochorionic, diamniotic. Two of the 10 monozygotic twins spontaneously converted to a singleton gestation.

Conclusions: Blastocyst transfer has been available for many years using co-culture, but there have been no published multicentre reports of monozygotic twinning associated with unmanipulated blastocysts. Sequential, cell-free media systems are relatively new. While the success with these systems has been substantial, until now, only the positive experiences from blastocyst transfer have been reported using these new media. In-vitro culture of embryos has been postulated to cause zona pellucida hardening. Extended in-vitro culture may cause increased hardening of the zona, which could potentially lead to pinching of the inner cell mass upon spontaneous hatching. Alternatively, current generation extended culture media may cause perturbations of cell-to-cell adhesion, facilitating splitting of the inner cell mass. Finally, a potentiating effect from the embryo transfer itself cannot be excluded. Whatever the mechanism, an increased incidence of monozygotic twinning following BET seems to be a real phenomenon, and should be taken into account when counselling patients.

P-082. Endometriosis and in-vitro fertilization: a meta-analysis

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Introduction: IVF has been used as a treatment alternative for women suffering from endometriosis for many years. However, there is no consensus as to whether the outcome of IVF is different in patients with the disease as compared to