

zonae pellucidae, whereas the amount of ZP2 appeared normal. Furthermore, there was an extra band in the 140 kDa region for the thick zonae pellucidae.

Conclusions: These data characterize a syndrome of thick zonae pellucidae which may be responsible for hypofertility. Further studies are needed to investigate whether the infertility is linked to an abnormal composition of the zona pellucida protein content. The infertility of the four sisters, leading to their inclusion in assisted fertilization programmes, suggests that it may have a genetic origin.

P089. Does 'turnover' of patients in IVF programmes influence the pregnancy rate?

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All physicians working in IVF centres receive patients disappointed by previously unsuccessful attempts at assisted reproduction and looking for a different centre to help finally achieve a pregnancy. In our centre, the numbers of patients who have already undergone one or more unsuccessful IVF attempts elsewhere have increased in the last few years to nearly 50% of cases.

Materials and methods: This retrospective study included 255 couples who underwent our IVF protocol from January 1993 to December 1995. The mean female age was 33.4 years (range 26–42) and the mean duration of infertility was 4.7 years (range 2–10). IVF indications were tubal factor 116 (45.5%), male factor 120 (47.1%), endometriosis stages III and IV (American Fertility Society) seven (2.7%) and unexplained infertility 12 (4.7%). All patients were treated following a standard stimulation protocol (GnRH_a/HMG). They were divided into two groups: group A included 123 patients asking for IVF as a first attempt and group B included 132 patients who had already undergone IVF trials elsewhere (at least three previous attempts). There was no significant difference (Student's unpaired *t*-test) between groups A and B concerning maternal age, the number of HMG ampoules, the oestradiol concentrations on the day of HCG administration, the number of oocytes retrieved and the number of embryos transferred.

Treatments in groups A and B (mean ± SD)

Group	Age (years)	No. of ampoules of HMG	Oestradiol concentration (pg/ml)	No. of oocytes	No. of embryos
A	32.64 ± 3.79	48.35 ± 16.50	1600.00 ± 1019.31	10.43 ± 5.77	1.83 ± 1.75
B	34.01 ± 3.34	50.20 ± 17.92	1510.00 ± 770.26	8.94 ± 5.06	1.94 ± 1.80
	(NS)	(NS)	(NS)	(NS)	(NS)

NS = not significant.

Results: We obtained 29 clinical pregnancies, with a cumulative pregnancy rate per cycle of 11.3%. In all, 24 pregnancies were achieved in patients aged ≤34 years (82.7%). We recorded 25 pregnancies in group A (no previous IVF attempts) out of 74 embryo transfers (pregnancy rate 33.8%). Four

pregnancies were achieved in group B (three or more previous IVF attempts) out of 79 embryo transfers (pregnancy rate 6.0%). Therefore the majority of pregnancies were achieved in patients not from other sterility centres.

Conclusions: Our data, according to the literature, confirm the critical relationship between age and pregnancy rate in IVF. Moreover, because no significant difference was found relating to age, response to ovulation induction, the number of oocytes recovered and the number of embryos transferred between the two groups considered, we can assume that, if a patient has already had at least three previously unsuccessful IVF attempts, the prognostic impact for subsequent attempts is very discouraging. Pregnancy rate will decrease markedly, according to our data to 6%.

P090. ICSI on metaphase I oocytes matured *in vitro*

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Introduction: In gonadotrophin-stimulated cycles, a proportion of the retrieved oocytes are not in the metaphase II stage at the time of oocyte collection. However, the majority of metaphase I oocytes will undergo full maturation *in vitro* within 24 h.

Materials and methods: Our aim was to compare the fertilization rate of injected metaphase II and metaphase I oocytes recovered in 145 cycles of IVF in our centre between March and December 1995.

Results: A total of 1662 oocytes were enzymatically denuded of cumulus cells with a solution of Flushing medium (Medicult, Denmark) containing 80 IU hyaluronidase/ml. Corona radiata cells were removed by aspiration in to and out of a fire-polished pipette. Of the 1662 oocytes, 1154 were in the metaphase II stage (69.4%), 254 were in the metaphase I stage (15.3%), 143 were in the germinal vesicle stage (8.6%) and 111 were abnormal (fractured zona, atretic) (6.7%). Of the 1154 metaphase II oocytes, 1051 were injected with ejaculated spermatozoa and 944 (89.8%) survived the ICSI procedure without damage. Of them, 636 (67.4%) showed normal fertilization (i.e. two distinct pronuclei 18 h after injection). Of the 254 metaphase I oocytes which had matured after 8 h of culture *in vitro*, 87 were also injected, and 77 of them (88.5%) survived. The fertilization rate was 42.9% (33 oocytes showing two pronuclei).

Conclusion: The fertilization rate, but not the degeneration rate, after the ICSI procedure was higher in metaphase II oocytes than in metaphase I oocytes matured *in vitro*. Taking into account this result, and the fact that it has not yet been evaluated whether ICSI in metaphase I oocytes involves increased risks, our policy is to inject metaphase I oocytes only when the number of metaphase II oocytes recovered per patient is three or less.