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Elevated serum progesterone on the day of HCG administration in IVF is associated with a higher pregnancy rate in polycystic ovary syndrome

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Our study compared 84 patients with polycystic ovary syndrome (PCOS) with 84 control patients who had normal ovaries and who were matched for the main determinants of success in in-vitro fertilization (IVF) and embryo transfer. Serum concentrations of oestradiol and progesterone on the day of human chorionic gonadotrophin (HCG) injection were significantly higher in PCOS than in normal patients (oestradiol 2016 ± 1.8 pg/ml versus 1456 ± 40.9 pg/ml, $P < 0.01$; progesterone 1.6 ± 0.1 ng/ml versus 1.2 ± 0.1 ng/ml, $P = 0.03$). Furthermore despite oocytes from PCOS patients having a reduced fertilization rate compared with normal patients (61.8 ± 4.1% versus 73.5 ± 4.3%, $P = 0.03$), the differences in pregnancy rate (22.6 versus 19%) and miscarriage (31.5 versus 18.7%) were not statistically significant. In PCOS patients, a critical breakpoint was identified at serum progesterone concentrations of 1.2 ng/ml on the day of HCG injection. The PCOS patients with progesterone ⩾1.2 ng/ml showed a higher pregnancy and miscarriage rate than PCOS patients with progesterone <1.2 ng/ml (26.6 versus 17.9%, $P < 0.01$; and 41.7% versus 14.3%, $P < 0.01$ respectively). These findings suggest that premature progesterone production does not have an adverse effect on pregnancy rate in PCOS, but on the contrary, may be a predictor for success in IVF/embryo transfer.

Key words: in-vitro fertilization/oestradiol/polycystic ovary syndrome/progesterone

Introduction

Polycystic ovary syndrome (PCOS) is the endocrine disorder most frequently associated with anovulation and repeated spontaneous abortion (Erickson and Yen, 1993; Fedele and Bianchi, 1995). For women with PCOS, failure to conceive can be treated with clomiphene citrate followed by treatment with gonadotrophins. In-vitro fertilization (IVF) and embryo transfer provide an additional line of treatment for PCOS patients who fail to conceive with follicle stimulating hormone (FSH) alone or in combination with gonadotrophin-releasing hormone agonist (GnRHa). There have been very few studies comparing the response of patients with or without PCOS to ovarian stimulation for IVF (Salat-Baroux *et al*., 1988; Dor *et al*., 1990; MacDougall *et al*., 1993; Homburg *et al*., 1993). Several investigators have confirmed that a serum progesterone concentration >0.9 ng/ml on the day of human chorionic gonadotrophin (HCG) injection is associated with a reduced pregnancy rate in patients undergoing IVF (Silveberg *et al*., 1991; Schoolcraft *et al*., 1991; Mio *et al*., 1992; Kagawa *et al*., 1992; Franchin *et al*., 1993; Silveberg *et al*., 1994). However, Hoffman *et al*. (1993, 1996) observed no significant difference in pregnancy rate in patients undergoing IVF/embryo transfer with high or low progesterone concentrations on the day of HCG administration and in patients who received oocytes donated from women with high or low progesterone concentrations. Furthermore, Legro *et al*. (1993) demonstrated that premature luteinization as detected by elevated serum progesterone is associated with higher pregnancy rate in donor oocyte in IVF.

In the present study we retrospectively evaluated the outcome of IVF in patients with and without PCOS and the effect of an elevated progesterone concentration on the day of HCG administration on pregnancy rate.

Materials and methods

Patients

This retrospective study analysed the results of 84 patients with normal ovaries (control) and 84 patients with PCOS participating in an IVF programme in the University of Milan, San Raffaele Scientific Institute, Reproductive Endocrinology Center, OB/GYN Clinic, Italy. All patients had failed to conceive in four ovulatory cycles after ovulation induction with gonadotrophins. They had all been investigated by hysterosalpingography and some of them also by laparoscopy and hysteroscopy. All of the male partners had normal semen quality according to World Health Organization (WHO, 1992) criteria. A pure tubal factor was the cause of infertility in the control patients, whereas anovulation was the only cause for PCOS patients. PCOS were classified according to menstrual history, hirsutism, hormonal concentrations and ultrasound examination. PCOS was diagnosed on the basis of having the following criteria: a history of anovulatory infertility and/or oligomenorrhoea or amenorrhoea, a Ferriman–Gallwey score >7 for hirsutism, hyperandrogenaemia, elevated concentrations of luteinizing hormone (LH) or with a LH/FSH ratio of 2 or more, increased ovarian volume (>9 ml), and 10 or more follicles of 2–8 mm in diameter. Only two PCOS patients were considered to be overweight, although none was characteristically obese. Normal patients had a history of normal menstrual cycles, normal ovarian size and no more than five follicles >2 mm in diameter.

Table I. Clinical and hormonal data from normal patients and patients with polycystic ovary syndrome (PCOS). Values are shown as means ± SE

	PCOS	Normal	Significance
Patients (*n*)	84	84	
Age (years)	32.7 ± 0.7	33.4 ± 0.4	NS
Body mass index	23.5 ± 0.9	21.2 ± 0.8	0.04
FSH concentration (mIU/ml)	5.7 ± 0.5	5.9 ± 0.4	NS
LH concentration (mIU/ml)	8.4 ± 0.9	5.1 ± 0.5	$P < 0.01$
Testosterone concentration (nmol/l)	4.5 ± 0.7	1.7 ± 0.3	$P < 0.01$
Androstenedione concentration (nmol/l)	13.8 ± 0.7	5.3 ± 0.7	$P < 0.01$

NS = not significant; FSH = follicle stimulating hormone; LH = luteinizing hormone.

Table II. Characteristics of in-vitro fertilization (IVF) cycles in both normal patients and patients with polycystic ovary syndrome (PCOS). Values are shown as means ± SE

	PCOS	Normal	Significance
Patients (*n*)	84	84	
Cycles (*n*)	104	116	
Total no. of ampoules of FSH	23.4 ± 1.8	30.3 ± 2.5	$P = 0.02$
Day of HCG administration	9.8 ± 0.5	11.5 ± 0.3	$P = 0.03$
Oestradiol concentration (pg/ml) at HCG	2016 ± 98.4	1456 ± 40.9	$P < 0.01$
Progesterone concentration (pg/ml) at HCG	1.6 ± 0.1	1.2 ± 0.1	$P = 0.03$
No. of follicles ⩽10 mm in diameter	4.8 ± 0.5	2.2 ± 0.3	$P < 0.01$
No. of follicles 10–15 mm in diameter	7.6 ± 0.7	4.3 ± 0.4	$P < 0.01$
No. of follicles ⩾16 mm in diameter	5.4 ± 0.4	3.3 ± 0.3	$P < 0.01$
No. of oocytes	11.5 ± 0.7	7.8 ± 0.2	$P < 0.01$
Fertilization rate (%)	61.8 ± 4.1	73.5 ± 4.3	$P = 0.03$
Mean no. of embryos transferred	2.3 ± 0.4	2.3 ± 0.3	NS
Pregancy rate/embryo transfer (%)	22.6 (19)	19.0 (16)	NS
Miscarriages (%)	31.5 (6)	18.7 (3)	NS

NS = not significant.

Table III. Characteristics of in-vitro fertilization (IVF) cycles in patients with polycystic ovary syndrome (PCOS) and varying progesterone concentrations. Values are shown as means ± SE

	Progesterone <1.2 ng/ml	Progesterone ⩾1.2 ng/ml	Significance
Patients (*n*)	39	45	
Cycles (*n*)	53	63	
Total no. of ampoules of FSH	43.8 ± 4.2	45.0 ± 2.4	NS
Day of HCG administration	10.2 ± 0.4	45.0 ± 2.4	NS
Oestradiol concentration (pg/ml) at HCG	1781 ± 125	2246 ± 146	$P < 0.01$
No. of oocytes	11.6 ± 1.0	12.1 ± 1.0	NS
Fertilization rate (%)	60.4 ± 3.7	62.4 ± 3.9	NS
Mean no. of embryos transferred	2.3 ± 0.4	2.3 ± 0.3	NS
Pregancy rate/embryo transfer (%)	17.9 (7)	26.6 (12)	$P < 0.01$
Miscarriages (%)	14.3 (1)	41.7 (5)	$P < 0.01$

NS = not significant.

Treatment protocol

All women in the IVF programme received a standard stimulation consisting of three ampoules per day of FSH (urofollitrophin, Metrodin; Serono, Rome, Italy) for 5 days. Adjustments of the dose of FSH were based on the individual dose response scheme whereby the dose was raised by one ampoule per day every 5 days until a follicular growth response and an increase in oestradiol concentration were noted. Final maturation of the oocytes was effected with 5000 IU HCG (Profasi; Serono, Rome, Italy) when there were at least two follicles >16 mm. Ovum retrieval was performed 32–36 h after HCG administration by vaginal ultrasound and embryo transfer 48 h later. The GnRHa employed was buserelin (Suprefact; Hoechst, L'Aquila, Italy) at a dose of 200 μg three times/day via intranasal spray. In women with PCOS, GnRHa was administered 3 weeks after a progestin-induced withdrawal bleed or spontaneous menstruation. In the control group, GnRHa was administered on days 20–21 of the cycle. At 2 weeks after GnRHa the serum concentration of oestradiol was measured and ultrasound examination of the ovaries performed. If the oestradiol concentration was <50 pg/ml and there were no follicles or cysts >10 mm diameter, gonadotrophin treatment was started. If this ovarian quiescence had not been achieved, a further examination was performed 1 week later.

Statistical analysis

All results are reported as the mean ± SE. Differences in the mean values for individual hormone measurements were assessed by using

Table IV. Characteristics of in-vitro fertilization (IVF) cycles in women with normal ovaries and varying concentrations of progesterone. Values are shown as means ± SE

	Progesterone <1.2 ng/ml	Progesterone ⩾1.2 ng/ml	Significance
Patients (*n*)	43	41	
Cycles (*n*)	54	50	
Total no. of ampoules of FSH	53.7 ± 4.2	48.5 ± 3.5	NS
Day of HCG administration	10.4 ± 0.4	10.2 ± 0.3	NS
Oestradiol concentration (pg/ml) at HCG	1406 ± 123	1698 ± 130	NS
No. of oocytes	5.8 ± 0.6	9.0 ± 0.9	$P < 0.01$
Fertilization rate (%)	74.8 ± 4.0	70.7 ± 3.2	NS
Mean no. of embryos transferred	2.2 ± 0.4	2.4 ± 0.3	NS
Pregancy rate/embryo transfer	16.3 (7)	24.4 (10)	NS
Miscarriages (%)	28.6 (2)	10.0 (1)	NS

NS = not significant.

analysis of variance and two-tailed group *t*-test. Serum progesterone breakpoints were optimized by summing the true positive rate and the true negative rate for each potential progesterone thresold from 0–1.8 ng/ml in increments of 0.1 ng/ml. This method is a variation of receiver operating characteristic (ROC) curve analysis (Hanely and McNeil, 1982). $P < 0.05$ was considered to be statistically significant.

Results

There were no significant differences in age and FSH of the patients with PCOS compared with those with normal ovaries (Table I). Body mass index (BMI), LH and androgen (testosterone and androstenedione) concentrations were, as expected, significantly raised ($P = 0.04$ and $P < 0.01$ respectively) in the PCOS patients compared with those in the controls (Table I). Despite receiving significantly fewer ampoules of follicle stimulating hormone FSH ($P = 0.02$) with fewer days of stimulation ($P = 0.03$), peak serum oestradiol concentrations were significantly higher ($P < 0.01$) in the PCOS group compared with the controls (Table II). Serum concentrations of progesterone on the day of HCG injection (35 h before follicular aspiration) were significantly higher ($P = 0.03$) in PCOS than in control patients (Table II). Furthermore, patients with PCOS developed significantly more follicles on the day of HCG administration ($P < 0.01$) compared with patients with normal ovaries irrespective of the size of the follicles and more oocytes were collected in the patients with PCOS (Table II). The mean fertilization rate in the patients with PCOS was 61.8 ± 4.1 compared with 73.5 ± 4.3 in the controls and this difference was statistically significant ($P = 0.03$) (Table II). The mean number of embryos transferred in both groups was similar (2.3 ± 0.4 and 2.3 ± 0.3) (Table II). There was a total of 19 (22.6%) pregnancies in the PCOS patients compared with 16 (19%) in the control group. Of these, six (31.5%) miscarried in the PCOS group compared with three (18.7%) in the control group. This difference was not significant (Table II). No patients in either group developed severe ovarian hyperstimulation syndrome following their IVF cycle.

In PCOS patients a critical breakpoint of 1.2 ng/ml was identified in serum progesterone concentrations on the day of HCG administration (Figure 1). PCOS patients were thus divided into two groups according their progesterone concentrations: <1.2 ng/ml and ⩾1.2 ng/ml (Table III). The PCOS patients with progesterone ⩾1.2 ng/ml ($n = 45$) had significantly higher serum oestradiol on the day of HCG administration with higher pregnancy rate and more miscarriages than did those with progesterone <1.2 ng/ml ($n = 39$). The differences of total number of FSH ampoules, day of HCG administration, number of oocytes obtained, fertilization rate and number of embryos transferred were not statistically significant between the two groups. In the control group the number of oocytes obtained was higher in patients with progesterone ⩾1.2 ng/ml than in those with progesterone <1.2 ng/ml and this was the only statistically significant difference (Table IV).

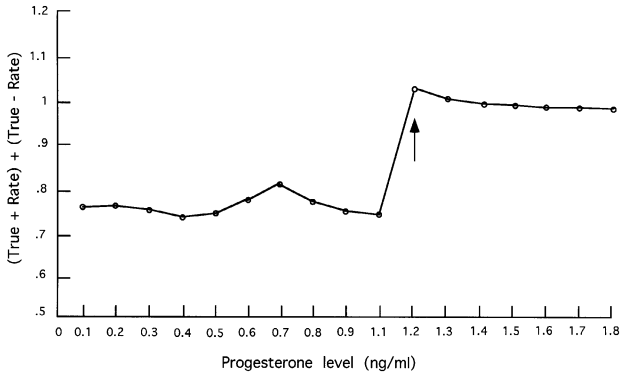

Figure 1. The effects of different threshold values of progesterone on the predictive value of pregnancy in patients with polycystic ovary syndrome (PCOS). The true positive rate (achieving a pregnancy if below the threshold) and the true negative rate (not achieving a pregnancy if above the threshold) were summed for each potential threshold value from 0–1.8 ng/ml in increments of 0.1 ng/ml.

Discussion

In the present study we have compared patients with PCOS and control patients who had normal ovaries and who matched for the main determinants of success in IVF. We found that: (i) despite a reduced fertilization rate in oocytes from PCOS patients compared with normal patients, the difference in pregnancy rate and and miscarriage were not statistically

significant; (ii) serum concentrations of oestradiol and progesterone on the day of HCG injection are significantly higher in PCOS patients; and (iii) using ROC analysis in PCOS patients, a critical breakpoint on the day of HCG injection was identified at progesterone concentration of 1.2 ng/ml and PCOS patients with progesterone ⩾1.2 ng/ml showed a higher pregnancy rate.

The response of patients with PCOS to ovulation induction is different from normal subjects (Hamilton-Fairley and Franks, 1990). Patients with PCOS have a greater number of follicles and oocytes, and reduced fertilization rate compared with patients with normal ovaries, but no difference in pregnancy rate and miscarriage (Dor *et al*., 1990; MacDougall *et al*., 1993). In agreement with these studies we found significantly higher peak serum oestradiol concentrations, number of follicles on the day of HCG injection, and number of oocytes collected. Oocytes from PCOS patients have reduced fertilization rate due to increased number of small and medium sized follicles. It is generally recognized that oocytes from relatively immature follicles have a lower fertilization rate compared with those from pre-ovulatory follicles.

Furthermore, in our study we confirmed that there is not a statistically significant difference in pregnancy rate and miscarriage. In a previous study that has been done on a smaller number of patients, we showed that serum progesterone on the day of HCG administration is significantly higher in PCOS compared with normal patients that undergo IVF cycles (Doldi *et al*., 1998). The significance of elevated progesterone concentrations at the time of HCG administration is not clearly known and opinion is somewhat divided. Several investigators have confirmed that high progesterone is associated with decreased success in IVF/embryo transfer cycles (Schoolcraft *et al*., 1991; Silveberg *et al*., 1991; Mio *et al*., 1992; Kagawa *et al*., 1992; Franchin *et al*., 1993; Silveberg *et al*., 1994). It has been suggested that this might be due to marked elevations in serum LH concentrations associated with elevated serum progesterone concentrations, rather than to premature progesterone production (Kagawa *et al*., 1992). Studies by Harada *et al.* (1995, 1996) showed that a rise in progesterone occurs even in patients with a combination of GnRH analogue and human menopausal gonadotrophin (HMG) without concomitant significant increase in immunoreactive LH and bioactive LH. These results, together with others (Silveberg *et al*., 1991; Schoolcraft *et al*., 1991), suggest that an endogenous LH rise may not be associated with these progesterone rises. It is more likely that luteinization occurred because of the excessive sensitivity of granulosa cells, rather than in response to excessive LH stimulation. Furthermore, Silveberg *et al*. (1991) observed a significantly greater fertilization rate in oocytes obtained from cycles in which progesterone concentration was >0.9 ng/ml compared with oocytes obtained from low progesterone cycles.

Therefore, it appears that premature progesterone production does not exert its adverse effect at the level of the oocyte. On the other hand Hoffman *et al*. (1996) observed no significant difference in pregnancy rate in patients undergoing IVF/embryo transfer cycles with high or low progesterone concentrations on the day of HCG injection. Furthermore, Legro *et al*. (1993) demonstrated that premature luteinization as detected by elevated serum progesterone is associated with high pregnancy rate with donor oocytes in IVF. In our study the difference in progesterone production between normal and PCOS patients on the day of HCG administration had no impact on pregnancy rate and miscarriage. Using a breakpoint of serum progesterone level of 0.9 ng/ml on the day of HCG injection (data not shown) as suggested by numerous published reports (Schoolcraft *et al*., 1991; Mio *et al*., 1992; Kagawa *et al*., 1992; Franchin *et al*., 1993), we found that PCOS patients with progesterone ⩾0.9 ng/ml showed a higher pregnancy rate. Using ROC analysis a critical breakpoint was identified at progesterone level of 1.2 ng/ml that is associated with pregnancy outcome. PCOS patients with progesterone ⩾1.2 ng/ml showed a higher pregnancy rate. Silveberg *et al*. (1994) observed that embryos obtained from IVF cycles in which premature progesterone production occurred, resulted in pregnancy at least as often as did embryos obtained from IVF in which premature progesterone production did not occur, with a trend toward a higher pregnancy rate with premature progesterone production.

Furthermore, previous studies, including our study, demonstrated that in-vivo and in-vitro progesterone production of PCOS granulosa cells is abnormal (Erickson *et al*., 1992; Doldi *et al*., 1998). Gilling-Smith *et al*. (1994) showed that in theca cells of PCOS ovaries, the androstenedione to progesterone ratio is significantly higher suggesting increased conversion of progesterone to androstenedione. Based on this information and on our results it seems that premature progesterone production does not have an adverse effect on pregnancy rate in PCOS, but on the contrary, may be a predictor for success in IVF/embryo transfer.

References

Doldi, N., Gessi, A., Destefani, A. *et al*. (1998) Polycystic ovary syndrome: anomalies in progesterone production. *Hum. Reprod.*, **13,** 290–293.

Dor, J., Shuman, A., Levran, D. and Ben-Rafael, Z. (1990) The treatment of patients with polycystic ovarian syndrome by in-vitro fertilization and embryo transfer: comparison of results with those patients with tubal infertility. *Hum. Reprod.*, **5,** 816–818.

Erickson, G.F., Magoffin, D.A., Garzo, V.G. *et al*. (1992) Granulosa cells of polycystic ovaries: are they normal or abnormal? *Hum. Reprod.*, **7,** 293–299.

Erickson, G.F., and Yen, S.S.C. (1993) The polycystic ovary syndrome. In Adashi, E.Y. and Leung, P.C.K. (eds), *The Ovary.* Raven Press, New York, USA, p. 561.

Fedele, L. and Bianchi, S. (1995) Habitual abortion: endocrinological aspects. [Review.] *Curr. Opin. Obstet. Gynecol.*, **7,** 351–356.

Franchin, R., de Ziegler, D., Taieb, J. *et al*. (1993) Premature elevation of plasma progesterone alters pregnancy rates of *in vitro* fertilization and embryo transfer. *Fertil. Steril.*, **59,** 1090–1094.

Gilling-Smith, C., Willis, D.S., Mason, H.D. and Franks, S. (1994) Hypersecretion of androstenedione by isolated thecal cells from polycystic ovaries. *J. Clin. Endocrinol. Metab.*, **79,** 1158–1165.

Hamilton-Fairley, D. and Franks, S. (1990) Common problems in induction of ovulation. *Ballière's Clin. Obstet. Gynaecol.*, **4,** 609–625.

Hanely, J.A. and McNeil, B.J. (1982) The meaning and use of the area under a receiver operating chracteristic (ROC) curve. *Radiology,* **143,** 29–34.

Harada, T., Yoshida, S., Katagiri, C. *et al*. (1995) Reduced implantation rate with subtle rise in serum progesterone concentration during the follicular phase of cycles stimulated with a combination of a gonadotropin-releasing hormone agonist and gonadotropin. *Hum. Reprod.*, **10,** 1060–1064.

Harada, T., Katagiri, C., Takao, N. *et al*. (1996) Altering the timing of human chorionic gonadotropin injection according to serum progesterone concentrations improves embryo quality in cycles with subtle progesterone rise. *Fertil. Steril.*, **65,** 594–597.

Hoffman, G.E., Bentzien, F., Bergh, P.A. *et al*. (1993) Premature luteinization

in controlled ovarian hyperstimulation has no adverse effect on oocyte and embryo quality. *Fertil. Steril.*, **60,** 675–679.

Hoffman, G.E., Khoury, J., Johnson, C.A. *et al*. (1996) Premature luteinization during controlled ovarian hyperstimulation for *in vitro* fertilization-embryo transfer has no impact on pregnancy outcome. *Fertil. Steril.*, **66,** 980–986.

Homburg, R., Berkowitz, D., Levy, T. *et al*. (1993) *In vitro* fertilization and embryo transfer for the treatment of infertility associated with polycystic ovary syndrome. *Fertil. Steril.*, **60,** 858–863.

Kagawa, T., Yamano, S., Nishida, S. *et al*. (1992) Relationship among serum levels of luteinizing hormone, estradiol, and progesterone during follicle stimulation and results of *in vitro* fertilization and embryo transfer (IVF/ET). *J. Assist. Reprod. Genet.*, **9,** 106–112.

Legro, R.S., Ary, B.A., Paulson, R.J. *et al*. (1993) Premature luteinization as detected by elevated serum progesterone is associated with a higher pregnancy rate in donor oocyte in-vitro fertilization. *Hum. Reprod.*, **8,** 1506–1511.

MacDougall, M.J., Tan, S.L., Balen, A. and Jacobs, H.S. (1993) A controlled study comparing patients with and without polycystic ovaries undergoing in-vitro fertilization. *Hum. Reprod.*, **8,** 233–237.

Mio, Y., Sekijima, A., Iwabe, T. *et al*. (1992) Subtle rise in serum progesterone during the follicular phase as a predictor of the outcome of *in vitro* fertilization. *Fertil. Steril.*, **58,** 159–166.

Salat-Baroux, J., Alvarez, S., Antoine, J.M. *et al*. (1988) Results of IVF in the treatment of of polycystic ovary disease. *Hum. Reprod.*, **3,** 331–335.

Schoolcraft, W., Sinton, E., Schenkler, T. *et al*. (1991) Lower pregnancy rate with premature luteinization during pituitary suppression with leuprolide acetate. *Fertil. Steril.*, **55,** 563–566.

Silveberg, K.M., Burns, W.N., Olive, D.L. *et al*. (1991) Serum progesterone levels predict success of *in vitro* fertilization/embryo transfer in patients stimulated with leuprolide acetate and human menopausal gonadotropins. *J. Clin. Endocrinol. Metab.*, **73,** 797–803.

Silveberg, K.M., Martin, M., Olive, D.L. *et al*. (1994) Elevated serum progesterone levels on the day of human chorionic gonadotropin administration in *in vitro* fertilization cycles do not adversely affect embryo quality. *Fertil. Steril.*, **61,** 508–513.

World Health Organization (1992) *WHO Laboratory Manual for the Examination of Human Semen and Sperm–Cervical Mucus Interaction.* Cambridge University Press, Cambridge, UK.

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