Article

Factors affecting live birth rate in intrauterine insemination cycles with recombinant gonadotrophin stimulation

Ahmet Erdem MD is associate professor of Obstetrics and Gynecology at the University of Gazi, Turkey. He is a graduate of the University of Hacettepe Medical School in Turkey and completed his residency at University of Gazi School of Medicine Department of Obstetrics and Gynecology in Ankara, Turkey. Since 1998, he has also been a clinician and researcher at the Assisted Reproductive Technologies Unit of the University of Gazi Center for Reproductive Health, Infertility and Family Planning. His research activity focuses on clinical IVF, intrauterine insemination and ovarian reserve testing.

Dr Ahmet Erdem

Ahmet Erdem[1], Mehmet Erdem, Songul Atmaca, Umit Korucuoglu, Onur Karabacak
University of Gazi School of Medicine, Department of Obstetrics and Gynecology, Besevler, Ankara, Turkey
[1]Correspondence: e-mail: Fax: +90 312 215 7736; erdemah@hotmail.com

Abstract

The objective of this cross-sectional study was to identify the prognostic factors that influence the outcome of ovarian stimulation with intrauterine insemination (IUI) cycles using gonadotrophins in couples with unexplained and mild male-factor subfertility. A total of 838 cycles in 456 women with unexplained and mild male-factor subfertility attending a university-based infertility clinic was evaluated. Of these cycles, 139 resulted in pregnancy (16.6% per cycle) and 96 out of 98 ongoing pregnancies resulted in live term birth. Live birth rate per patient and per cycle was 21.1% and 11.4%, respectively. Multivariate logistic regression analysis demonstrated that duration of infertility ($P = 0.034$), type of infertility ($P = 0.003$), aetiology of infertility ($P = 0.004$), number of treatment cycles ($P = 0.0001$) and number of dominant follicles before human chorionic gonadotrophin (HCG; $P = 0.024$) were significant independent factors to predict clinical pregnancy. The duration of infertility ($P = 0.043$), number of treatment cycles ($P = 0.0001$) and number of dominant follicles before HCG ($P = 0.024$) were significant independent factors to predict live birth. In conclusion, for subfertile couples having shorter duration of subfertility, multifollicular response to gonadotrophins and in their first treatment cycle are more likely to succeed a live birth with IUI treatment using recombinant gonadotrophins.

Keywords: *clinical pregnancy, intrauterine insemination, live birth rate, male factor, recombinant gonadotrophins, unexplained subfertility*

Introduction

Approximately 15–30% of infertile couples have unexplained and male-factor subfertility (Guzick *et al.*, 1994; Smith *et al.*, 2003; Collins and van Steirteghem, 2004). The treatment algorithm in such patients includes timely coitus, ovarian stimulation, ovarian stimulation with intrauterine insemination (IUI) and other complex assisted reproduction techniques (Practice Committee of the American Society for Reproductive Medicine, 2006). Ovarian stimulation with IUI forms the baseline treatment in couples with mild-to-moderate male-factor and unexplained infertility (Guzick *et al.*, 1998; Practice Committee of the American Society for Reproductive Medicine, 2006). IUI is a non-invasive and inexpensive technique compared with other treatments. The main steps of ovarian stimulation and IUI include the stimulation of patients with either clomiphene citrate or gonadotrophins and sperm preparation with swim-up or gradient techniques. The chance of spontaneous pregnancy per cycle for couples with unexplained infertility is 1.3–4.1%, and it increases to 10.5–17.9% when patients are treated with IUI with or without ovarian stimulation (Guzick *et al.*, 1998, 1999).

Several factors influence the effectiveness of IUI. The use of IUI appears to improve cycle fecundity when combined with ovarian stimulation, and a pregnancy rate between 4% and 40% after ovarian stimulation and IUI has been reported in patients with unexplained and male-factor infertility using

clomiphene citrate or gonadotrophins (Karlström *et al.*, 1993; Fanchin *et al.*, 1995; Brzechffa *et al.*, 1998; Nuojua-Huttunen *et al.*, 1999; Steures *et al.*, 2004; Custers *et al.*, 2007). In several studies, it has also been demonstrated that the outcome of IUI is dependent on age, presence of a male factor, duration and cause of infertility and the number of pre-ovulatory follicles in ovarian stimulation before insemination (Kerin *et al.*, 1994; Tomlinson *et al.*, 1996; Tummon *et al.*, 1997; Cohlen *et al.*, 1998; Sahakyan *et al.*, 1999; Stone *et al.*, 1999; Hendin *et al.*, 2000; Khalil *et al.*, 2001; Van Voorhis *et al.*, 2001; Ahinko-Hakamaa *et al.*, 2007).

Despite the many studies that have been performed to assess the prognostic value of these variables in predicting the outcome of IUI, there are many methodological shortcomings that prevent the drawing of definitive conclusions. These prognostic variables have been assessed in a few studies by multivariate analysis (Tomlinson *et al.*, 1996; Nuojua-Huttunen *et al.*, 1999; Khalil *et al.*, 2001; Steures *et al.*, 2004) and in only one study has a prediction model been used to make a distinction between patients with a good or poor prognosis (Custers *et al.*, 2007). In the majority of studies, the main outcome measure was clinical pregnancy, which is not such a relevant standard of success in reproductive medicine as live birth rate per cycle. Apart from this, the study populations included heterogeneous groups with regard to aetiology and ovarian stimulation protocols, in which clomiphene citrate and many types of gonadotrophins were used in the same study group with diverse aetiologies, including unexplained and male infertility as well as tubal factor, anovulation and endometriosis. Indeed, it has been shown that addition of gonadotrophins to IUI was more effective than clomiphene citrate in patients with unexplained and male-factor subfertility (Practice Committee of the American Society for Reproductive Medicine, 2006).

In view of these issues, this study aimed to investigate the effect of factors including female age, aetiology, type and duration of infertility, sperm motility before and after sperm preparation, and the number of dominant follicles before administration of human chorionic gonadotrophin (HCG) to the success of IUI cycles in patients with unexplained and mild male-factor subfertility, where all patients were uniformly treated with recombinant gonadotrophins.

Materials and methods

Patients and study protocol

In this cross-sectional study, 838 cycles of ovarian stimulation IUI treatment with recombinant FSH (rFSH) in 456 patients with unexplained and mild male infertility were analysed from December 1999 to March 2007. Inclusion criteria for couples with unexplained subfertility were as follows: infertility duration of at least 1 year; regular menstrual cycles with mid-luteal progesterone concentrations >10 ng/ml; bilateral tubal patency confirmed with hysterosalpingography and/or laparoscopy; and normal semen analysis according to World Health Organization criteria (World Health Organization, 1993). Patients having the female criteria for inclusion but with sperm count ≤20 × 10^6/ml and/or sperm motility ≤50% was regarded as mild male infertility, and included in the study. Patients with polycystic ovary syndrome, previous ovarian surgery, one ovary or with a single patent uterine tube at hysterosalpingography and/or laparoscopy and with total progressive motile sperm count <1 × 10^6/ml after sperm preparation were excluded from the study. Patients who had minimal endometriosis at laparoscopy, and lesions either were ablated or removed, were included in this study. This retrospective study was carried out in accordance with local ethics committee regulations.

Semen preparation

Semen samples were taken by masturbation and collected in sterile containers. After liquefaction of the fresh ejaculate semen for 30 min at room temperature, each sample was analysed for volume, concentration, and motility using World Health Organization criteria (World Health Organization, 1993). Swim-up was the preferred method for semen preparation. In the swim-up technique, one-quarter of the semen was taken into a tube, and sperm medium containing human serum albumin (SpermRinse; VitroLife, Kungsback, Sweden) was added to the semen in a 1:1 proportion. The supernatant was separated after 10 min of centrifugation at 287 *g* and 0.25 ml of sperm medium was added over the pellet at a 45° angle. Incubation was then performed at 37°C for 1 h, with the angle of inclination kept constant. After the procedure, sperm concentration, sperm motility and total motile sperm number were evaluated, and the inseminate was stored in an incubator at 37°C until the time of insemination.

Ovarian stimulation, insemination procedure and detection of pregnancy

Patients were treated with 75 IU rFSH (Puregon; Organon, Istanbul, Turkey and Gonal-F; Serono, Istanbul, Turkey) beginning from day 3 of the cycle and ovarian response was assessed with transvaginal ultrasonography (TVU) from day 8. Gonadotrophin doses were adjusted for each patient individually according to the follicular development. Ovulation was triggered with 10,000 IU HCG (Pregnyl; Organon) when at least one dominant follicle had reached 18 mm in diameter. As minimal stimulation with a low starting dose of recombinant gonadotrophins was used, ovarian response was not monitored routinely with oestradiol measurement. In selected cases at high risk of ovarian hyperstimulation syndrome, cycles were cancelled if oestradiol concentrations were higher than 1500 pg/ml. IUI was performed 36 h after HCG administration with a disposable IUI catheter (Gynetics; Gynetics Medical Products, Achel, Belgium or Embryon; Rocket Medical, Tyne and Wear, Washington, UK). The patient rested in a supine position for 15 min after the procedure. All patients had luteal support with micronized progesterone vaginal gel (Crinone 8%; Serono) at a dose of 90 mg per day.

Pregnancy testing was performed by determining the quantitative serum HCG concentration at 14 days after HCG administration, and intrauterine pregnancy was confirmed by TVU at 2 weeks after a positive pregnancy test. A clinical pregnancy was defined as the presence of a gestational sac on TVU or by histological examination of products of conception in patients who aborted. Live birth was defined as having a child who was living at 1 week after birth. Cycles, if any, that did not progress to IUI because of hyper-response or no response to ovarian stimulation were not included in the analysis.

Outcome measures and statistical analysis

The primary outcome measures were clinical pregnancy and live birth. The Statistical Program for Social Sciences (SPSS, version 11.5; SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Data were expressed as mean ± SD. Comparisons of mean age, duration of infertility, basal FSH and basal oestradiol, sperm number and total sperm motility before and after sperm preparation, total progressive motile sperm number after sperm preparation and the number of dominant follicles >16 mm before HCG administration between cycles with or without clinical pregnancy and live birth were performed using the unpaired *t*-test. Comparisons of infertility diagnosis (primary or secondary) and aetiology (unexplained or male) between cycles with or without clinical pregnancy and live birth were performed using chi-squared test. Univariate and multivariate logistic regression analyses were used to determine the effect of significant variables as determinants of clinical pregnancy and live birth. A logistic regression model was estimated by using the block entry of variables. Selection of variables was performed with a significance level of 5%. Duration of infertility, type of infertility (primary versus secondary), aetiology of infertility (unexplained versus male), sperm number before swim-up, sperm motility before and after swim-up, number of treatment cycles (one cycle versus two, three, or four or more cycles), and the number of dominant follicles before HCG (one follicle versus two, three, or four or more follicles) were the variables used in regression model. $P < 0.05$ was considered statistically significant.

Results

A total of 456 patients underwent 838 cycles of ovarian stimulation IUI. Of these, 325 (71.3%) patients had primary infertility and 278 (61.0%) patients had unexplained infertility. Mean infertility duration of the patients was 4.7 ± 3.8 years (range, 1–18 years). Mean age of the patients was 29.7 ± 5.1 (range, 19–45 years). Of 456 patients, 82 patients with unexplained infertility and 27 patients with male-factor infertility had a history of laparoscopy. Sixteen patients with unexplained infertility and one patient with male-factor infertility had minimal endometriosis. All lesions were ablated with electrocoagulation.

Of the 456 patients, 238 had one treatment cycle, 116 had two, 63 had three, and 39 had four or more cycles. Mean number of cycles per patient was 1.83.

Follicular responses to ovarian stimulation of 838 cycles were as follows. In 54.7% of cycles there was one dominant follicle, there were two dominant follicles in 29.4%, three dominant follicles in 9.8% and four or five dominant follicles in 6.1% of cycles. As follicular response to ovarian stimulation with gonadotrophins was one of the main variables analysed in this study to predict clinical pregnancy and live birth, cycles with four or five dominant follicles with oestradiol concentrations <1500 pg/ml were not cancelled. Ten cycles were cancelled due to hyper-response and high oestradiol values on the day of HCG administration. No cycle was cancelled as a result of low ovarian response.

Of the 838 cycles, 139 resulted in pregnancy (16.6% per cycle). Clinical pregnancy rate per patient was calculated as 26.5%. Ten pregnancies were biochemical (7.2%) and 31 pregnancies (22.3%) resulted in spontaneous abortion. Abortion rate per pregnancy was 22.4%. Of the 98 ongoing pregnancies, 96 resulted in a live term birth. Ongoing pregnancy rate per patient and per cycle was 21.5% and 11.6%, respectively. Live birth rate per patient and per cycle was 21.1% and 11.4%, respectively. Twenty-eight (6.1%) patients had spontaneous pregnancies between cycles, and all pregnancies except two progressed to term: 16 were after the first cycle, four were after the second cycle, six were after the third cycle, and two pregnancies were after the fourth and the fifth cycles. Including spontaneous pregnancies, which ended in live birth, cumulative live birth rate per patient was 26.7%. Out of 98 ongoing pregnancies, there were seven (7.1%) twin gestations and four (4.1%) high-order gestations (one triplet, three quadruplets). Only two multiple gestations (one twin and one quadruplet) did not reach term.

When the occurrence of multiple pregnancies was analysed in comparison to the number of dominant follicles on the day of HCG injection, only four of 11 multiple pregnancies were achieved in cycles with more than one dominant follicles (three set of twins and one set of quadruplets) whereas seven others were achieved in cycles with only one dominant follicle. In two patients with multiple pregnancies, there was only one dominant follicle and two intermediate follicles with a diameter of 9–16 mm, and in the other patients the number of intermediate follicles was between three and six.

Live birth rates per patient in couples with primary and secondary infertility were 18.8% and 29.3%, respectively, and the difference was statistically significant ($P < 0.05$). Live birth rate per patient was significantly higher in couples with unexplained infertility (25.2%) compared with male-factor infertility (15, 7%), ($P < 0.05$).

Tables 1 and **2** show the differences in clinical parameters with regard to clinical pregnancy and live birth. Mean duration of infertility was significantly lower in cycles that resulted in clinical pregnancy and live birth, compared to non-pregnant cycles ($P = 0.022$ and $P = 0.014$ respectively). There was a statistically significant difference between clinical pregnancy rate per cycle in couples with primary and secondary infertility ($P = 0.006$). There was also a statistically significant difference between clinical pregnancy and live birth rates per cycle in couples with unexplained infertility compared to male-factor infertility ($P = 0.0001$ and $P = 0.003$, respectively). Sperm number and motility before swim-up and sperm motility after swim-up were significantly lower in non-pregnant cycles compared to cycles that did result in clinical pregnancy ($P = 0.033$, $P = 0.0001$ and $P = 0.002$ respectively), whereas only sperm motility before swim-up was significantly higher in cycles that resulted in live birth ($P = 0.003$). The number of dominant follicles on the day of HCG administration was significantly higher in cycles that resulted in clinical pregnancy and live birth compared to non-pregnant cycles ($P = 0.005$ and $P = 0.009$ respectively).

Table 3 shows univariate and multivariate logistic regression analysis and effects of variables to predict clinical pregnancy and live birth in ovarian stimulation IUI cycles. Univariate logistic regression analysis revealed that duration of infertility

Table 1. The effect of duration, type and aetiology of infertility, female and sperm parameters and dominant follicle numbers on clinical pregnancy rates.

Parameter	*Pregnant*	*Non-pregnant*	P-*value*
Duration of infertility (years)	4.1 ± 2.9	4.8 ±3.6	0.022
Age (years)	29.3 ± 5.1	30.2 ± 5.2	NS
Type of infertility *n* (%)			
Primary	81 (13.3)	527 (86.7)	0.006
Secondary	48 (20.9)	182 (79.1)	
Aetiology of infertility *n* (%)			
Unexplained	100 (20.3)	392 (79.7)	0.0001
Male	29 (8.4)	317 (91.6)	
Basal FSH (mIU/ml)	6.9 ± 2.9	7.5 ± 4.2	NS
Basal oestradiol (pg/ml)	43.0 ± 19.6	55.1 ± 48.4	NS
Sperm number before swim-up (×10^6)	60.7 ± 42.9	51.9 ± 41.8	0.033
Sperm motility before swim-up (%)	60.2 ± 16.7	53.4 ± 16.4	0.0001
Sperm number after swim-up (×10^6)	49.8 ± 45.0	43.2 ± 43.8	NS
Sperm motility after swim-up (%)	84.5 ± 13.0	79.0 ± 17.9	0.002
Total progressive motile sperm number after swim-up (×10^6)	41.2 ± 42.3	35.6 ± 39.5	NS
Number of dominant follicles before HCG	2.1 ± 1.2	1.7 ± 1.1	0.005

Values are mean ± SD unless otherwise stated. HCG = human chorionic gonadotrophin; NS = not statistically significant.

Table 2. The effect of duration, type and aetiology of infertility, female and sperm parameters and dominant follicle numbers on live birth rates.

Parameter	*Pregnancy with live birth*	*Non-pregnant or pregnancy loss*	P-*value*
Duration of infertility (years)	3.9 ± 2.8	4.8 ±3.6	0.014
Age (years)	29.5 ± 4.7	30.1 ± 5.2	NS
Type of infertility *n* (%)			
Primary	62 (10.2)	546 (89.8)	NS
Secondary	34 (14.8)	196 (85.2)	
Aetiology of infertility *n* (%)			
Unexplained	69 (14.0)	423 (86.0)	0.003
Male	27 (7.8)	319 (92.2)	
Basal oestradiol (pg/ml)	43.1 ± 15.7	54.4 ± 47.5	NS
Sperm number before swim-up (×10^6)	59.5 ± 42.9	52.5 ± 41.9	NS
Sperm motility before swim-up (%)	59.2 ± 16.2	53.8 ± 16.6	0.003
Sperm number after swim-up (×10^6)	50.0 ± 48.6	43.4 ± 43.4	NS
Sperm motility after swim-up (%)	82.9 ± 13.3	79.3 ± 17.8	NS
Total progressive motile sperm number after swim-up (×10^6)	41.2 ± 42.3	35.6 ± 39.5	NS
Number of dominant follicles before HCG	2.1 ± 1.3	1.7 ± 1.1	0.009

Values are mean ± SD unless otherwise stated. HCG = human chorionic gonadotrophin; NS = not statistically significant.

Table 3. Univariate and multivariate logistic regression analysis of different variables to predict clinical pregnancy and live birth in intrauterine insemination cycles.

Variable	*Clinical pregnancy*			*Live birth*		
	OR	*95% CI*	P-*value*	*OR*	*95% CI*	P-*value*
Univariate analysis						
Duration of infertility	0.932	0.88–0.99	0.025	0.912	0.847–0.983	0.015
Type of infertility (primary versus secondary)	1.716	1.15–2.54	0.007	–	–	–
Aetiology of infertility (unexplained versus male)	0.358	0.231–0.555	0.0001	0.530	0.334–0.840	0.007
Sperm number before swim-up	1.005	1.00–1.009	0.035	–	–	–
Sperm number after swim-up	1.003	0.999–1.007	NS	–	–	–
Sperm motility before swim-up	1.027	1.014–1.040	0.0001	1.021	1.007–1.035	0.004
Sperm motility after swim-up	1.023	1.009–1.037	0.001	–	–	–
No. of treatment cycles						
One	0.575	0.478–0.692	0.0001	0.419	0.325–0.541	0.0001
Two	0.424	0.268–0.671	0.0001	0.337	0.202–0.564	0.0001
Three	0.399	0.299–0.534	0.0001	0.342	0.244–0.479	0.0001
Four or more	0.484	0.385–0.609	0.0001	0.375	0.281–0.500	0.0001
No. of dominant follicles before HCG						
One	1.312	1.105–1.558	0.002	1.351	1.121–1.628	0.002
Two	1.245	0.792–1.957	NS	1.027	0.610–1.729	NS
Three	1.426	1.079–1.884	0.013	1.149	0.919–1.742	NS
Four or more	1.551	1.209–1.889	0.0001	1.504	1.175–1.925	0.001
Multivariate analysis						
Duration of infertility	0.930	0.870–0.995	0.034	0.925	0.858–0.998	0.043
Type of infertility (primary versus secondary)	1.989	1.255–3.154	0.003	–	–	–
Aetiology of infertility (unexplained versus male)	0.420	0.233–0.757	0.004	0.654	0.374–1.146	NS
Sperm number before swim-up	1.0	0.993–1.006	NS	–	–	–
Sperm motility before swim-up	–	–	–	1.012	0.995–1.028	NS
Sperm motility after swim-up	1.003	0.984–1.021	NS	–	–	–
Number of treatment cycles	0.613	0.507–0.742	0.0001	0.467	0.361–0.604	0.0001
Number of dominant follicles before HCG (≥3)	1.774	1.079–2.917	0.024	1.786	1.046–3.05	0.033

CI = confidence interval; HCG = human chorionic gonadotrophin; NS = not statistically significant.

(P = 0.025), type of infertility (P = 0.007), aetiology of infertility (P = 0.0001), sperm number before swim-up (P = 0.035), sperm motility before swim-up (P = 0.0001), sperm motility after swim-up (P = 0.001), number of treatment cycles (P = 0.0001), number of dominant follicles before HCG (one dominant follicle versus three follicles, P = 0.013; and one follicle versus four or more follicles, P = 0.0001) were the factors that significantly predicted clinical pregnancy with IUI. With multivariate analysis, only duration of infertility (P = 0.034), type of infertility (P = 0.003), aetiology of infertility (P = 0.004), number of treatment cycles (P = 0.001) and number of dominant follicles before HCG (P = 0.024) were significant independent factors to predict clinical pregnancy with IUI. When the effects of variables to predict live birth were analysed on univariate analysis, duration of infertility (P = 0.015), aetiology of infertility (P = 0.007), sperm motility before swim-up (P = 0.004), number of treatment cycles (P = 0.0001) and number of dominant follicles before HCG (one follicle versus four or more follicles, P = 0.001) were the factors that significantly predicted live birth in IUI cycles. With multivariate analysis, duration of infertility (P = 0.043), number of treatment cycles (P = 0.0001) and number of dominant follicles before HCG (P = 0.033) were significant independent factors to predict live birth in IUI cycles.

Cycles with a diagnosis of unexplained infertility were also analysed separately with regard to the impact of outcome variables to predict clinical pregnancy and live birth in ovarian stimulation and IUI cycles. There were no differences related to clinical, hormonal and sperm parameters with regard to clinical pregnancy except for sperm motility before swim-up, which was higher in cycles that did result in clinical pregnancy

as compared with non-pregnant cycles (data not shown). There was a statistically significant difference between clinical pregnancy rates per cycle in couples with primary infertility (64 out of 355 cycles; 18.0%) and secondary infertility (36 out of 137 cycles; 26.3%), ($P < 0.01$). With multivariate analysis, only type of infertility [odds ratio (OR), 1.728, 95% confidence interval (CI) = 1.032–2.895, $P = 0.038$], number of treatment cycles (one cycle versus two, three, or four or more cycles, OR = 0.717, 95% CI = 0.588–0.874, $P = 0.001$), number of dominant follicles before HCG (one dominant follicle versus three or more follicles, OR = 1.856, 95% CI = 1.075–3.207, $P = 0.027$) were the factors that significantly predicted clinical pregnancy in ovarian stimulation and IUI cycles in patients with unexplained infertility.

Mean duration of infertility was significantly lower in cycles that did result in live birth, as compared with non-pregnant cycles (4.9 ± 3.6 years versus 3.9 ± 2.9, respectively, $P < 0.05$) in patients with unexplained infertility. The number of dominant follicles on the day of HCG administration was significantly higher in cycles that did result in a live birth as compared with non-pregnant cycles (2.1 ± 1.4 versus 1.8 ± 1.2, respectively, $P < 0.01$). Other clinical, hormonal and sperm parameters with regard to live birth were not different (data not shown). The duration of infertility (OR = 0.898, 95% CI = 0.820–0.934, $P = 0.022$), the number of treatment cycles (one cycle versus two, three, or four or more cycles, OR = 0.518, 95% CI = 0.392–0.683, $P = 0.0001$), and number of dominant follicles before HCG (one dominant follicle versus three or more follicles, OR = 2.077, 95% CI = 1.138–3.792, $P = 0.017$) were the factors that significantly predicted live birth in ovarian stimulation and IUI cycles in patients with unexplained infertility.

Discussion

Most couples complaining of unexplained and mild male-factor infertility are in fact subfertile; they have decreased fecundity rates but natural conception is still possible (ESHRE Capri Workshop, 1996). IUI with ovarian stimulation is a simple and inexpensive treatment modality for subfertile couples with a mean pregnancy rate of 8.3% and 17.1% per cycle for clomiphene citrate plus IUI and human menopausal gonadotrophin plus IUI, respectively (Guzick *et al.*, 1998). In the current study, clinical pregnancy rate per cycle was 16.6% and live birth rate per cycle was 11.4%, which was higher than the chance of spontaneous pregnancy per cycle for couples with unexplained infertility (Guzick *et al.*, 1998). The success rates of ovarian stimulation and IUI cycles are affected by various factors, namely the duration of infertility, aetiology of infertility, maternal age, use of different stimulation protocols, number of dominant follicles and sperm parameters. These data demonstrated that duration of infertility ($P = 0.034$), number of treatment cycles ($P = 0.0001$) and number of dominant follicles before HCG ($P = 0.024$) were significant independent factors to predict live birth in ovarian stimulation and IUI cycles. Although type (primary versus secondary) and aetiology (unexplained versus mild male factor) of infertility was important to predict clinical pregnancy, they were not associated with prognosis when live birth was used as primary outcome measure.

There are no data from well-designed prospective studies that investigate prognostic factors in IUI cycles in couples with the diagnosis of only unexplained infertility. On the other hand, if data from retrospective series is processed, multivariate logistic regression analysis is an important statistical method of investigating prognostic factors in IUI cycles. However, multivariate analysis has been used to assess the outcome of ovarian stimulation and IUI with regard to prognostic factors in some (Tomlinson *et al.*, 1996; Nuojua-Huttunen *et al.*, 1999; Khalil *et al.*, 2001; Van Voorhis *et al.*, 2001; Steures *et al.*, 2004; Ahinko-Hakamaa *et al.*, 2007), but not all studies (Sahakyan *et al.*, 1999; Stone *et al.*, 1999; Dickey *et al.*, 2002). Despite the large number of studies that have been performed, only one study has used ongoing pregnancy as an outcome variable as compared with clinical pregnancy and found that increasing maternal age, longer duration of infertility as well as presence of male infertility, one-sided tubal pathology and endometriosis were unfavourable predictors, whereas the use of ovarian stimulation and cervical factor were favourable predictors for an ongoing pregnancy (Steures *et al.*, 2004). In several other studies, the duration of infertility (Tomlinson *et al.*, 1996; Nuojua-Huttunen *et al.*, 1999; Iberico *et al.*, 2004), number of cycles (Nuojua-Huttunen *et al.*, 1999; Khalil *et al.*, 2001; Steures *et al.*, 2004), and number of dominant follicles before HCG administration (Tomlinson *et al.*, 1996; Nuojua-Huttunen *et al.*, 1999; Stone *et al.*, 1999; Khalil *et al.*, 2001; Montanaro Gauci *et al.*, 2001; Iberico *et al.*, 2004; Ahinko-Hakamaa *et al.*, 2007) were also found to affect the success rates of ovarian stimulation and IUI cycles, in concordance with this study.

In the current study, male factor has a negative impact to predict only clinical pregnancy, but it was not associated with outcome when live birth was taken as the outcome measure. Sperm parameters also did not predict outcome with multivariate analysis. The lack of association of sperm parameters as variables in multivariate analysis with treatment outcome might be explained by the effective increase in the number of motile sperm after the swim-up procedure. The lack of association of male-factor infertility as a determinant of outcome with multivariate analysis might also be explained by the fact that some mild male-factor patients interfere with unexplained infertility; that is to say that some patients who are classified as mild male-factor infertility have normal sperm counts with a minor decrease in motility, and should in fact be regarded as unexplained. In this series, patients had isolated asthenospermia, without low sperm counts and motility, in 45.9% of cycles. In fact, multivariate analysis in the subgroup of patients with unexplained infertility in this study revealed that duration of infertility, number of treatment cycles and number of dominant follicles before HCG were the factors that predicted live birth. This indicates that a diagnosis of mild male-factor infertility does not have a negative impact on live birth when these patients were treated with ovarian stimulation and IUI as with unexplained infertility.

Unexplained infertility and anovulation have been identified as favourable factors to predict the likelihood of pregnancy as compared with tubal factor and endometriosis (Nuojua-Huttunen *et al.*, 1999; Khalil *et al.*, 2001; Montanaro Gauci *et al.*, 2001; Dickey *et al.*, 2002; Steures *et al.*, 2004). In the current study, the aetiology of infertility has been verified in all patients with hysterosalpingography and/or diagnostic laparoscopy; thus, patients with previous ovarian surgery, with one ovary or with a single patent uterine tube were excluded. Although patients with mild endometriosis were included in the unexplained infertility group, they were not treated as a

subgroup in the analysis, as the number of patients was small (3.7% of all patients) and their lesions were ablated during laparoscopy. In fact, the exact incidence of endometriosis could not be estimated, unless the diagnosis of unexplained infertility has been verified by laparoscopy in all cases. This reflects one of the major limitations of retrospective analyses, including the current study.

Female ageing causes a decrease in the number of ovarian follicles, resulting in a poor response to the ovulation induction treatment and low pregnancy rates after ovarian stimulation and IUI cycles (Sahakyan *et al*., 1999). However, age of the female partner was not found to be a prognostic factor to predict outcome in ovarian stimulation and IUI cycles in current study. Although evidence from several studies suggests that age is a major determinant of IUI success (Campana *et al*., 1996; Stone *et al*., 1999; Hendin *et al*., 2000; Khalil *et al*., 2001; Montanaro Gauci *et al*., 2001; Steures *et al*., 2004), some other studies have failed to find this association in patients younger than 40 years, in concordance with this study (Tomlinson *et al*., 1996; Nuojua-Huttunen *et al*., 1999). The mean age of the patients in this study was 29.7 years, which represents a younger population. This might be the main factor, which explains why age did not affect success rates of ovarian stimulation and IUI cycles. In fact, it has been shown that the impact of age on the success of IUI is more prominent on the extremes of reproduction. It has been demonstrated that success with IUI is reduced significantly in women older than 40 years (Frederick *et al*., 1994; Haebe *et al*., 2002). Age, therefore, does not affect success rates of IUI cycles in a relatively younger female population.

The number of dominant follicles before HCG administration, a cycle characteristic, was associated with outcome in various studies (Tomlinson *et al*., 1996; Nuojua-Huttunen *et al*., 1999; Stone *et al*., 1999; Khalil *et al*., 2001; Montanaro Gauci *et al*., 2001; Iberico *et al*., 2004; Ahinko-Hakamaa *et al*., 2007). In the current study, the number of dominant follicles before HCG was significant when more than three follicles were compared with single follicle development (clinical pregnancy rate, $P = 0.024$; live birth rate, $P = 0.033$). It has been reported in other studies that presence of three or more dominant follicles is associated with a two- to three-fold increase in pregnancy rates compared with monofollicular growth in IUI cycles stimulated with clomiphene citrate or gonadotrophins (Nuojua-Huttunen *et al*., 1999; Dickey *et al*., 2002). It was also shown that addition of stimulation, especially with gonadotrophins, was more effective than only IUI in patients with unexplained and male-factor subfertility (Khalil *et al*., 2001; Steures *et al*., 2004; Practice Committee of the American Society for Reproductive Medicine, 2006). However, the use of different stimulation protocols and gonadotrophins for ovulation induction might potentially interfere with the influence of number of follicles on ovarian stimulation and IUI success.

One of the major drawbacks of ovarian stimulation with gonadotrophins in IUI cycles is the increased risk of multiple pregnancies. Although the contribution made by IUI to the number of multiple pregnancies is much smaller than the contribution made by IVF (Steures *et al*., 2007) and the risk of multiple pregnancy does not necessarily seem to affect the patients' preferences for IUI with ovarian stimulation (van Weert *et al*., 2007), there is a worldwide acceptance among healthcare providers to prevent increased risk of multiple pregnancies not only with IVF but also with IUI treatments. This study observed relatively low multiple pregnancy rates of 11.1% (7.1% twin and 4.1% high-order gestations), although multifollicular response to gonadotrophin stimulation was favourably associated with live birth rates. The multiple pregnancy rate observed in this study is in concordance with the results of a recent nationwide report from The Netherlands, in which multiple pregnancies were reported to occur in 9.5% of pregnancies achieved in IUI cycles (Steures *et al*., 2007). Cancellation of the cycles according to excess number of dominant follicles is one of the universally accepted strategies used to prevent multiple pregnancies in ovulation induction with gonadotrophins. Theoretically, if stimulation with gonadotrophins in IUI cycles has a beneficial effect on pregnancy rates, strict cancellation criteria of dominant follicle numbers in favour of lowering multiple pregnancy rates might reduce overall success. Interestingly, the majority of multiple pregnancies observed in this study was achieved in patients having one dominant but multiple intermediate follicles. In conclusion, the results of this study clearly show that the cohort of intermediate follicles in response to stimulation with gonadotrophins seems to be an important factor for the occurrence of multiple pregnancies.

In the current study, a few cycles were cancelled due to hyper-response to ovarian stimulation with gonadotrophins. Theoretically, it may be considered that analysis of the main outcome measures after excluding cancelled cycles will have a positive effect on the results. However, the effect of excluding cancelled cycles on the main outcome measures is considered to be negligible as the number of cancelled cycles was small (<0.1% of total cycles).

One of the important features of current study is that, although the study design is cross-sectional, all patients were treated similarly by ovarian stimulation and IUI with recombinant gonadotrophins in a prospective manner. Thus, it is suggested that the impact of cycle characteristics, such as the number of dominant follicles and treatment cycles, on the outcome of ovarian stimulation and IUI was better manifested in a homogenous study population using a unique stimulation protocol. The second and probably the most important feature of the current study is the use of live birth, the major and the ultimate aim of any fertility treatment, as an outcome measure in a multivariate regression analysis. In fact, some prognostic variables, such as type and aetiology of infertility, were not significant at all when outcome measure was live birth. This may partly explain the divergent impact of common prognostic variables in different study populations where clinical pregnancy was used as main outcome measure.

In conclusion, these results suggest that number of the treatment cycle, duration of infertility and multifollicular response are the most important prognostic factors in predicting live birth rate as a successful outcome in ovarian stimulation and IUI cycles with gonadotrophins in couples with unexplained and mild male-factor subfertility. However, the age of the female partner, infertility type (primary versus secondary) and aetiology (unexplained versus mild male), as well as sperm parameters, were not found to be prognostic factors to predict live birth in ovarian stimulation and IUI cycles. Although not prospective, data from this study might be helpful for better selection and counselling of couples with unexplained and mild male-factor infertility having ovarian stimulation and IUI treatment.

References

Ahinko-Hakamaa K, Huhtala H, Tinkanen H 2007 Success in intrauterine insemination: the role of etiology. *Acta Obstetrica Gynecologica Scandinavica* **86**, 855–860.

Brzechffa PR, Daneshmand S, Buyalos RP 1998 Sequential clomiphene citrate and human menopausal gonadotrophin with intrauterine insemination: the effect of patient age on clinical outcome. *Human Reproduction* **13**, 2110–2114.

Campana A, Sakkas D, Stalberg A *et al*. 1996 Intrauterine insemination: evaluation of the results according to the woman's age, sperm quality, total sperm count per insemination and life table analysis. *Human Reproduction* **11**, 732–736.

Cohlen BJ, te Velde ER, van Kooij RJ *et al*. 1998 Controlled ovarian hyperstimulation and intrauterine insemination for treating male subfertility: a controlled study. *Human Reproduction* **13**, 1553–1558.

Collins JA, Van Steirteghem A 2004 Overall prognosis with current treatment of infertility. *Human Reproduction Update* **10**, 309–316.

Custers IM, Steures P, van der Steeg JW *et al*. 2007 External validation of a prediction model for an ongoing pregnancy after intrauterine insemination. *Fertility and Sterility* **88**, 425–431.

Dickey RP, Taylor SN, Lu PY *et al*. 2002 Effect of diagnosis, age, sperm quality and number of preovulatory follicles on the outcome of multiple cycles of clomiphene citrate-intrauterine insemination. *Fertility and Sterility* **78,** 1088–1095.

Fanchin R, Olivennes F, Righini C *et al*. 1995 A new system for fallopian tube sperm perfusion leads to pregnancy rates twice as high as standard intrauterine insemination. *Fertility and Sterility* **64,** 505–510.

Frederick JL, Denker MS, Rojas A *et al*. 1994 Is there a role for ovarian stimulation and intrauterine insemination after age 40? *Human Reproduction* **9**, 2284–2286.

Guzick DS, Carson SA, Coutifaris C *et al*. 1999 Efficacy of superovulation and intrauterine insemination in the treatment of infertility. *New England Journal of Medicine* **340**, 177–183.

Guzick DS, Sullivan MW, Adamson GD *et al*. 1998 Efficacy of treatment for unexplained infertility. *Fertility and Sterility* **70**, 207–213.

Guzick DS, Grefenstette I, Baffone K *et al*. 1994 Infertility evaluation in fertile women: a model for assessing the efficacy of infertility testing. *Human Reproduction* **9**, 2306–2310.

Haebe J, Martin J, Tekepety F *et al*. 2002 Success of intrauterine insemination in women aged 40–42 years. *Fertility and Sterility* **78**, 29–33.

Hendin BN, Falcone T, Hallak J *et al*. 2000 The effect of patient and semen characteristics on live birth rates following intrauterine insemination: a retrospective study. *Journal of Assisted Reproduction and Genetics* **17**, 245–252.

Iberico G, Vioque J, Ariza N *et al*. 2004 Analysis of factors influencing pregnancy rates in homologous intrauterine insemination. *Fertility and Sterility* **81,** 1308–1313.

Karlström PO, Bergh T, Lundkvist O 1993 A prospective randomized trial of artificial insemination versus intercourse in cycles stimulated with human menopausal gonadotropin or clomiphene citrate. *Fertility and Sterility* **59,** 554–559.

Kerin JFP, Peek J, Warnes GM *et al*. 1994 Improved conception rates after intrauterine insemination of washed spermatozoa from men with poor quality semen. *Lancet* **310**, 533–534.

Khalil MR, Rasmussen PE, Erb K *et al*. 2001 Homologous intrauterine insemination. An evaluation of prognostic factors based on a review of 2473 cycles. *Acta Obstetrica Gynecologica Scandinavica* **80**, 74–81.

Montanaro Gauci M, Kruger TF, Coetzee K *et al*. 2001 Stepwise regression analysis to study male and female factors impacting on pregnancy rate in an intrauterine insemination programme. *Andrologia* **33**, 135–141.

Nuojua-Huttunen S, Tomas C, Bloigu R *et al*. 1999 Intrauterine insemination treatment in subfertility: an analysis of factors affecting outcome. *Human Reproduction* **14,** 698–702.

Practice Committee of the American Society for Reproductive Medicine 2006 Effectiveness and treatment for unexplained infertility. *Fertility and Sterility* **86**, S111–S114.

Sahakyan M, Harlow BL, Hornstein MD 1999 Influence of age, diagnosis, and cycle number on pregnancy rates with gonadotropin-induced controlled ovarian hyperstimulation and intrauterine insemination. *Fertility and Sterility* **72**, 500–504

Smith S, Pfeifer SM, Collins JA 2003 Diagnosis and management of female infertility. *Journal of American Medical Association* **290**, 1767–1770.

Steures P, van der Steeq JW, Hompes PG *et al*. 2007 Intrauterine insemination in The Netherlands. *Reproductive BioMedicine Online* **14**, 110–116.

Steures P, van der Steeg JW, Mol BW *et al*. CECERM (Collaborative Effort in Clinical Evaluation in Reproductive Medicine) 2004 Prediction of an ongoing pregnancy after intrauterine insemination. *Fertility and Sterility* **82**, 45–51.

Stone BA, Vargyas JM, Ringler GE *et al*. 1999 Determinants of outcome of intrauterine insemination: analysis of outcomes of 9963 consecutive cycles. *American Journal of Obstetrics and Gynecology* **180**, 1522–1534.

The ESHRE Capri Workshop 1996 Guidelines to the prevalence, diagnosis, treatment and management of infertility. *Human Reproduction* **11,** 1775–1807.

Tomlinson MJ, Amissah-Arthur JB, Thompson KA *et al*. 1996 Prognostic indicators for intrauterine insemination: statistical model for IUI success. *Human Reproduction* **11,** 1892–1896.

Tummon LS, Asher LJ, Martin JS *et al*. 1997 Randomized controlled trial of superovulation and insemination for infertility associated with minimal or mild endometriosis. *Fertility and Sterility* **68**, 8–12.

van Weert JM, van den Broek J, van der Steeg JW *et al*. 2007 Patients' preferences for intrauterine insemination or in-vitro fertilization. *Reproductive BioMedicine Online* **15**, 422–427.

Van Voorhis BJ, Barnett M, Sparks AE *et al*. 2001 Effect of the total motile count on the efficacy and cost-effectiveness of intrauterine insemination and in-vitro fertilization, *Fertility and Sterility* **75**, 661–668.

World Health Organization 1993 *Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction*. Cambridge University Press, New York, 3rd ed.

Declaration: The authors report no financial or commercial conflicts of interest.

Received 28 November 2007; refereed 14 December 2007; accepted 26 March 2008.