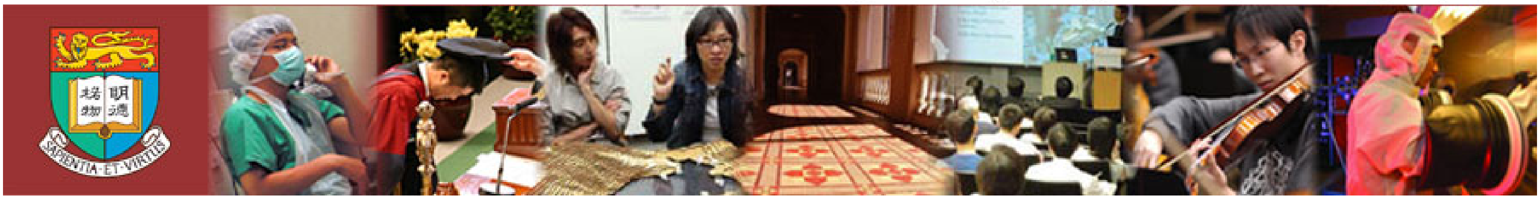

G Makkar
EHY Ng 吳鴻裕
WSB Yeung 楊樹標
PC Ho 何柏松

Prognostic factors for successful outcome in patients undergoing controlled ovarian stimulation and intrauterine insemination

通過促排卵與宮內授精而成功懷孕的預後因素

Objective. To determine the prognostic factors associated with successful outcome following controlled ovarian stimulation and intrauterine insemination.
Design. Retrospective analysis.
Setting. University-based assisted reproductive technology centre, Hong Kong.
Patients and methods. Patients included 292 couples undergoing 600 treatment cycles, following a standard protocol of human menopausal gonadotrophin injections. Multiple logistic regression analysis was performed to determine which demographic and sperm parameters gave the maximum discrimination to predict pregnancy.
Results. One hundred and eleven pregnancies resulted from treatment. The pregnancy rates were 18.5% per cycle and 37.9% per couple. The age of the women was significantly lower for pregnant cycles, and the serum oestradiol levels and number of follicles greater than 16 mm in diameter were significantly higher, compared with non-pregnant cycles. The sperm concentration and number of motile spermatozoa were also significantly increased in pregnant cycles. Pregnancy rate was significantly increased when the raw semen sample contained 20 million/mL or more spermatozoa, normal forms comprised 7% or more, and when the number of motile spermatozoa in inseminated samples was 1 million or greater.
Conclusion. Using multiple logistic regression analysis, age of the women and serum oestradiol level had the maximum power to predict pregnancy following ovarian stimulation and intrauterine insemination.

目的：確定通過促排卵與宮內授精而成功懷孕的預後因素。
設計：回顧研究。
安排：香港一所大學的輔助生育中心。
患者及方法：292對夫婦接受共600個療程，根據標準程序接受絕經後促性腺激素注射。研究利用多元對數回歸分析確定那些人口參數和精子參數能最有效地預測懷孕的機會。
結果：治療後共有111個成功懷孕的個案。妊娠率按療程計為18.5%，按每對夫婦計則為37.9%。與非懷孕週期比較，懷孕週期的女性年齡明顯較低，而血清雌二醇水平和直徑大於16微米的濾泡數量均明顯較高。精子濃度及能動精子的數量在懷孕週期也明顯增加。就精液樣本而言，若每毫升精液含精子2000萬或以上，其中7%或以上屬正常，或者能動精子的數量在用於受精的樣本達100萬或以上的話，妊娠率會明顯提高。
結論：利用多元對數回歸分析研究發現，經過促排卵與宮內授精後，女性年齡和血清雌二醇水平能最有效地預測懷孕的機會。

Key words:
Insemination;
Ovarian stimulation;
Prognosis;
Semen

關鍵詞：
授精；
促排卵；
預後；
精液

Hong Kong Med J 2003;9:341-5

Department of Obstetrics and Gynaecology, The University of Hong Kong, Queen Mary Hospital, Pokfulam, Hong Kong
G Makkar, MB, BS, MPhil
EHY Ng, MB, BS, FHKAM (Obstetrics and Gynaecology)
WSB Yeung, PhD
PC Ho, MD, FHKAM (Obstetrics and Gynaecology)

Correspondence to: Dr EHY Ng
(e-mail: nghye@hkucc.hku.hk)

Introduction

Intrauterine insemination (IUI) in conjunction with controlled ovarian stimulation (COS) is usually offered to infertile couples if the woman has patent fallopian tubes, prior to other assisted reproductive methods. Controlled ovarian stimulation may correct subtle problems of ovulation, increase the number of oocytes available for fertilisation, and enhance the accuracy of timing of insemination. The rationale for performing IUI is that a higher number of motile spermatozoa with normal forms can be inseminated around the time of ovulation to the uterine cavity, closer to the site of fertilisation. Moreover, sperm preparation removes leukocytes and dead and moribund spermatozoa from the semen sample. These can generate free oxygen radicals and reduce the functional capacity of intact spermatozoa.[1,2]

The aim of this retrospective analysis was to determine prognostic factors for successful outcome following COS/IUI.

Patients and methods

Six hundred COS/IUI cycles for 292 couples completed between February 1993 and August 2001 at the Department of Obstetrics and Gynaecology, Queen Mary Hospital, were analysed retrospectively. All women in this study had a diagnosis of infertility for more than 2 years, regular menstrual cycles, bilateral patent fallopian tubes shown on diagnostic laparoscopy, and no contra-indications for pregnancy. Patients with multiple causes of infertility or anovulation were excluded from the study.

All patients underwent COS using human menopausal gonadotrophin (HMG). On the second day of the treatment cycle, the serum oestradiol (E2) level was checked and baseline transvaginal scanning was performed. If E2 level was less than 220 pmol/L, and there was no ovarian cyst evident on the scan, treatment of 150 IU of HMG (Pergonal; Serono, Aubonne, Switzerland) was given intramuscularly daily from day 3 onwards. The ovarian response was regularly assessed using both transvaginal scanning and serum E2 levels. Ten thousand international units of human chorionic gonadotrophin (HCG) [Profasi; Serono, Aubonne, Switzerland] were given when the leading follicle was greater than 18 mm in diameter, and there were no more than three follicles of greater than 16 mm in diameter. Patients with an excessive response were counselled to have the cycle cancelled because of the increased risk of multiple pregnancy.

The husband was asked to submit a semen sample in a sterile plastic container about 2 hours before the IUI procedure, after an abstinence of 2 to 3 days. The sample was allowed to liquefy completely at room temperature, usually occurring within 30 minutes. After liquefaction, sperm preparation was completed by a discontinuous density gradient centrifugation method, using Percoll (Pharmacia, Uppsala, Sweden) or Isolate (Irvine Scientific, Santa Ana, US) sperm separation media.[3] The pellet obtained after centrifugation was washed twice with Earl's balanced salt solution (EBSS; Sigma, St Louis, US), supplemented with 0.35% Plasmanate (PPF; Bayer Corporation, Elkhart, US) or 8% patient's serum. The resulting sperm pellet after washing was overlaid with the same medium, adjusting the final volume to 0.3 mL to 0.5 mL.

Semen analysis was performed according to World Health Organization (WHO) guidelines,[4] both before and after sperm preparation. Concentration, progressive motility, and normal morphology were evaluated after staining by Diff-Quick method.[5] A thin and well-spread smear was air-dried on a clean glass slide at room temperature. The slides were fixed in Diff-Quick fixative (1.8 mg/L triarylmethane in methyl-alcohol) for 15 seconds, stained in Diff-Quick solution 1 (1 g/L xanthene in sodium acide-preserved buffer) for 10 seconds, and finally in Diff-Quick solution 2 (0.625 g/L azure A and 0.625 g/L methylene blue in buffer) for 10 seconds. Morphology was assessed by counting 100 spermatozoa using WHO criteria.[4]

Intrauterine insemination was performed once, 38 hours after HCG using a Tomcat catheter (Monoject, St Louis, US) as described previously.[6,7] The patient was asked to rest in the supine position for 15 minutes after the procedure, and thereafter to resume her routine activities. The luteal phase was supported by two further doses of 1500 IU HCG on day 5 and day 10, after the ovulatory HCG injection. Serum E2 and progesterone levels were also checked on day 10 after the ovulatory HCG injection. Pregnancy testing was performed on day 20 after the ovulatory HCG injection, and if positive, a pelvic ultrasound was arranged to confirm the presence of an intrauterine pregnancy and to determine the number of gestational sacs. In vitro fertilisation treatment was advised if the patient was not pregnant after three treatment cycles.

Statistical analysis

Only clinical pregnancies were considered in this retrospective analysis. A clinical pregnancy was defined as the presence of an intrauterine gestational sac(s) on scanning, or products of conception on histological examination in cases of miscarriage. Biochemical pregnancies were excluded from the analysis. The number of motile spermatozoa (in millions) was obtained by multiplying semen volume and the concentration and percentage of progressive motile spermatozoa. Recovery rate (%) was calculated by dividing the total motile spermatozoa in the prepared sample by that of the raw sample.

Data were expressed as median and range. Comparison of various characteristics in pregnant and non-pregnant cycles was carried out using the Mann-Whitney *U* test. Multiple logistic regression analysis was performed to determine which demographic and sperm parameters gave maximum discrimination to predict pregnancy. Statistical analysis was performed using the Statistical Package for the Social Sciences (Windows version 10; SPSS Inc., Chicago, US). Two-tailed $P<0.05$ values were considered statistically significant.

Results

One hundred and eleven pregnancies were achieved, and the pregnancy rate (PR) was 18.5% per treatment cycle, and

Table 1. Demographic characteristics and ovarian response in pregnant and non-pregnant cycles

	Pregnant cycles* (n=111)	Non-pregnant cycles* (n=489)
Causes of infertility		
Male factor	25	150
Endometriosis; unexplained	86	339
Age of women (years)	33.0 (25.0-37.0)†	34.0 (23.0-45.0)†
Duration of infertility (years)	3.0 (2.0-13.0)	4.0 (2.0-13.0)
Ampoules of human menopausal gonadotrophin given	14 (6-46)	14 (5-88)
No. of follicles >16 mm in diameter	2 (1-5)‡	1 (0-5)‡
Oestradiol level on ovulatory human chorionic gonadotrophin (pmol/L)	2770 (39-11134)§	1788 (70-12132)§
Size of the largest follicle (mm)	18.7 (16.0-25.0)	19.0 (16.0-28.0)

* Values reported are the median (range), unless otherwise specified
† P=0.033
‡ P=0.005
§ P<0.001

Table 2. Comparison of spermatozoa parameters in raw and inseminated semen between pregnant and non-pregnant cycles

	Raw semen			Prepared semen		
	Pregnant cycles*	Non-pregnant cycles*	P value	Pregnant cycles*	Non-pregnant cycles*	P value
Volume (mL)	3.2 (0.3-11.0)	3.2 (0.3-12.0)	NS†	0.53 (0.50-0.70)	0.54 (0.30-0.80)	NS
Concentration (million/mL)	75.0 (1.6-1040.0)	63.5 (3.0-500.0)	0.040	35.0 (0.2-265.0)	24.0 (0.4-480.0)	0.006
Progressive motility (%)	52.0 (5.0-87.0)	51.0 (0-87.0)	NS	83.0 (36.0-96.0)	85.0 (0-98.0)	NS
No. of motile spermatozoa (million)	116.6 (0.2-1154.0)	93.7 (0.6-1083.0)	0.010	14.5 (0.04-144.0)	10.0 (0.02-259.0)	0.010
Normal morphology using WHO criteria (%)	20.0 (3.0-54.0)	18.0 (0-76.0)	0.020	41.0 (6.0-83.0)	35.0 (0-82.0)	0.049
Recovery rate (%)	-	-	-	13.3 (1.2-77.0)	11.2 (0.7-68.0)	NS

* Values reported are the median (range)
† NS not significant

37.9% per couple. The PR per cycle was 14.3% (25/175) for couples with male-factor infertility, and 20.2% (86/425) for other causes, including mild endometriosis and unexplained infertility. The multiple PR (twins and triplets) was 22.6%. There were 12 (10.8%) clinical abortions, and two (1.8%) ectopic pregnancies. A significantly higher PR was noted in the first (19.9%) and second (21.1%) cycles than in the third cycle (12.1%). None of the patients experienced moderate or severe ovarian hyperstimulation syndrome.

Table 1 summaries demographic data and ovarian response data. The median age of women was significantly lower in pregnant cycles than in non-pregnant cycles (33.0 years vs 34.0 years, P=0.03, Mann-Whitney *U* test). The median E2 level and the median number of follicles greater than 16 mm in diameter on the ovulatory HCG day were significantly higher in pregnant cycles compared to non-pregnant cycles.

The volume of semen, percentage of motile spermatozoa in the raw and inseminated samples, and the recovery rate of motile spermatozoa were similar in pregnant and non-pregnant cycles (Table 2). The sperm concentration, the percentage of normal spermatozoa, and the number of motile spermatozoa were significantly higher in pregnant than non-pregnant cycles in both raw and inseminated semen samples.

Table 3. Factors of prognostic significance on multiple logistic regression analysis

	Standard discriminant function coefficient β	P value
Age of women	-0.098	0.009
Oestradiol level on human chorionic gonadotrophin day	0.000	<0.001
Normal spermatozoa (raw sample)	0.026	0.011
Concentration (inseminated sample)	0.003	0.039

A significantly higher PR was achieved when the sperm concentration in the raw semen sample was 20 million/mL or greater (19.7% vs 9.2%, P<0.05, Chi squared test), or the percentage of normal morphology was 7% or greater (19.4% vs 10.3%, P<0.05, Chi squared test). When the number of motile spermatozoa in inseminated samples was 1 million or greater, PR was also significantly increased (19.4% vs 3.7%, P<0.05, Chi squared test). The percentage of progressive motility in both raw and inseminated samples did not affect PRs, even when it was as low as 10%.

Using logistic regression analysis, the serum E2 level on the day of HCG was the most discriminating variable to predict pregnancy following IUI (P<0.001) [Table 3]. The age of women showed a negative correlation with pregnancy (standard discriminant function coefficient β= –0.098, P=0.009). The log concentration of spermatozoa in the inseminated samples demonstrated a significant correlation with the outcome of insemination ($P<0.05$). The percentage of normal forms, and the concentration of spermatozoa in raw semen samples were other significant semen parameters predictive of pregnancy ($P<0.05$).

Discussion

Controlled ovarian stimulation/IUI treatment is a less expensive, less stressful, and less invasive treatment compared with other assisted reproduction techniques, such as in vitro fertilisation treatment. Consequently, it is usually the first-line treatment offered to patients with patent fallopian tubes and varying causes of infertility. Three stimulated cycles have been reported as necessary to optimise the success of IUI.[8,9] Our study showed a significantly higher PR in the first and second cycles, compared with the third cycle. Burr et al[10] have also reported a sharp decrease in PR from the first cycle to the third cycle. A reasonable chance of conception, however, is present even in the third cycle. Tomlinson et al[11] reported a success rate in the third cycle of 14%, in keeping with this study's findings. Other studies have also reported a higher PR in the first cycle, and that the majority of pregnancies are established within three to four cycles. It seems then that COS/IUI is most effective in the first three treatment cycles.

A higher number of follicles with a mean diameter of greater than 16 mm were present in pregnant compared to non-pregnant cycles. This was reflected in higher serum E2 level in pregnant cycles. A better PR has been reported in cycles with three follicles of greater than 16 mm in diameter,[12] as well as in cycles with two follicles of greater than 16 mm in diameter.[9] The risk of multiple pregnancy should be taken into consideration with respect to multi-follicular development, however.

This study found that the age of the women was a significant discriminating factor predictive of pregnancy. No pregnancy was achieved in women older than 38 years. Similar reports in the literature have noted that women older than 40 years had a poor success rate after IUI with ovarian stimulation treatment.[9,11-14] These findings are in keeping with other previous studies documenting a decline in female fecundity with donor spermatozoa.[15-18] This decline has been suggested to be a result of decreased oocyte quality,[19,20] higher rates of chromosomal abnormalities,[21] and/or reduced uterine receptivity.[22,23] Extensive counselling should be offered to women who are older than 38 to 40 years before they proceed to COS/IUI treatment. However, there have been some studies showing that the success of IUI does not decline with increasing female age.[11,24]

The influence of sperm parameters in predicting the results of assisted reproductive techniques is a matter of debate. In IUI, conventional semen parameters have been reported to have no power to discriminate between pregnant and non-pregnant ejaculates in either donor insemination[25] or IUI programmes.[13] Controversially, conventional andrological diagnosis has been said to be essential for IVF or insemination therapy.[26]

Sperm concentration and the number of motile spermatozoa in both raw and inseminated semen samples were significantly higher in pregnant than non-pregnant cycles in our study. Sperm concentration, progressive motility, and the number of motile spermatozoa have been reported to be significantly correlated with PR in IUI,[27] in donor insemination cycles,[18,28] and with the time of conception.[29] Similarly, Bielsa et al[30] reported that the number of motile spermatozoa inseminated showed the best association with fertility outcome ($P<0.003$). Conversely, no correlation was demonstrated between PR and inseminated number of motile spermatozoa by Burr et al,[10] and by Horvath et al[27] when the concentration of spermatozoa was between 1 and 30 million.

The chances of achieving pregnancy were reported to be significantly increased when the raw semen sample contained 20 million/mL or more spermatozoa, below which the relationship between fertilisation capacity and sperm density was said to be less predictable.[31] A similar threshold limit of approximately one million progressive motile spermatozoa for reasonable IUI success has previously been reported.[27,32,33] However, pregnancies have been achieved with IUI with less than one million progressive motile spermatozoa in washed samples during natural cycles,[8] with 0.8 million motile spermatozoa or more,[34] and with as low as 0.2 million motile spermatozoa.[10] Notwithstanding, the fact that the number of motile spermatozoa used for insemination influences the outcome of IUI, highlights the importance of using washing procedures that result in good levels of spermatozoa recovery.[3]

The percentage of normal morphology in raw and inseminated semen samples was significantly higher in pregnant compared with non-pregnant cycles. Moreover, the proportion of normal spermatozoa in the raw semen was shown to predict successful outcome. The percentage of normal morphology of raw and 'post swim-up' semen has also been found to correlate with in vitro fertilisation rates,[30,35-37] and with higher PRs in IUI[10,38] and donor insemination programmes.[39] The threshold value for normal morphology in raw semen was 7%, below which PRs were significantly reduced. It has been suggested that the minimum percentage of normal sperm in raw semen for IUI cycles should be 10%.[10]

Conclusion

Age of the women and E2 level had maximum power to predict pregnancy following COS and insemination, with lower age of women and higher E2 level correlating positively with successful outcome. An increased number of follicles of greater than 16 mm in diameter were present in pregnant cycles compared with non-pregnant cycles. In addition, sperm concentration and the number of motile spermatozoa were also significantly increased in pregnant cycles. The PR was significantly increased when the raw semen sample contained 20 million/mL or more spermatozoa, normal forms comprised 7% or greater, and when the number of motile spermatozoa in inseminated samples was 1 million or greater.

References

1. Aitken RJ, Clarkson JS. Significance of reactive oxygen species and antioxidants in defining the efficacy of sperm preparation techniques. J Androl 1988;9:367-76.
2. Vogelpoel FR, van Kooij RJ, te Velde ER, Verhoef J. Influence of polymorphonuclear granulocytes on the zona-free hamster oocyte assay. Hum Reprod 1991;6:1104-7.
3. Makkar G, Ng HY, Yeung SB, Ho PC. Comparison of two colloidal silica-based sperm separation media with a non-silica-based medium. Fertil Steril 1999;72:796-802.
4. World Health Organization (WHO) laboratory manual for the examination of human semen and sperm-cervical mucus interaction. 3rd ed. Cambridge: The Press Syndicate of the University of Cambridge; 1992.
5. Kruger TF, Ackerman SB, Simmons KF, Swanson RJ, Brugo SS, Acosta AA. A quick, reliable staining technique for human sperm morphology. Arch Androl 1987;18:275-7.
6. Ho PC, So WK, Chan YF, Yeung WS. Intrauterine insemination after ovarian stimulation as a treatment for subfertility because of subnormal semen: a prospective randomized controlled trial. Fertil Steril 1992; 58:995-9.
7. Makkar G, Ng EH, Yeung WS, Ho PC. A comparative study of raw and prepared semen samples from two consecutive days. J Reprod Med 2001;46:565-72.
8. Byrd W, Ackerman GE, Carr BR, Edman CD, Guzick DS, McConnell JD. Treatment of refractory infertility by transcervical intrauterine insemination of washed spermatozoa. Fertil Steril 1987;48:921-7.
9. Plosker SM, Jacobson W, Amato P. Predicting and optimizing success in an intra-uterine insemination programme. Hum Reprod 1994;9: 2014-21.
10. Burr RW, Siegberg R, Flaherty SP, Wang XJ, Matthews CD. The influence of sperm morphology and the number of motile sperm inseminated on the outcome of intrauterine insemination combined with mild ovarian stimulation. Fertil Steril 1996;65:127-32.
11. Tomlinson MJ, Amissah-Arthur JB, Thompson KA, Kasraie JL, Bentick B. Prognostic indicators for intrauterine insemination (IUI): statistical model for IUI success. Hum Reprod 1996;11:1892-6.
12. Nuojua-Huttunen S, Tomas C, Bloigu R, Tuomivaara L, Martikainen H. Intrauterine insemination treatment in subfertility: an analysis of factors affecting outcome. Hum Reprod 1999;14:698-703.
13. Hull MG, Eddowes HA, Fahy U, et al. Expectations of assisted conception for infertility. BMJ 1992;304:1465-9.
14. Frederick JL, Denker MS, Rojas A, et al. Is there a role for ovarian stimulation and intra-uterine insemination after age 40? Hum Reprod 1994;9:2284-6.
15. Byrd W, Bradshaw K, Carr B, Edman C, Odom J, Ackerman G. A prospective randomized study of pregnancy rates following intrauterine and intracervical insemination using frozen donor sperm. Fertil Steril 1990;53:521-7.
16. Stovall DW, Toma SK, Hammond MG, Talbert LM. The effect of age on female fecundity. Obstet Gynecol 1991;77:33-6.
17. van-Noord-Zaadstra BM, Looman CW, Alsbach H, Habbema JD, te Velde ER, Karbaat J. Delaying childbearing: effect of age on fecundity and outcome of pregnancy. BMJ 1991;302:1361-5.
18. Kang BM, Wu TC. Effect of age on intrauterine insemination with frozen donor sperm. Obstet Gynecol 1996;88:93-8.
19. Navot D, Bergh PA, Williams MA, et al. Poor oocyte quality rather than implantation failure as a cause of age-related decline in female fertility. Lancet 1991;337:1375-7.
20. Abdalla HI, Burton G, Kirkland A, et al. Age, pregnancy and miscarriage: uterine versus ovarian factors. Hum Reprod 1993;8:1512-7.
21. Richardson SJ, Nelson JF. Follicular depletion during the menopausal transition. Ann NY Acad Sci 1990;592:13-20,44-51.
22. Flamigni C. Egg donation to women over 40 years of age [editorial]. Hum Reprod 1993;8:1343-4.
23. Cano F, Simon C, Remohi J, Pellicer A. Effect of aging on the female reproductive system: evidence for a role of uterine senescence in the decline in female fecundity. Fertil Steril 1995;64:584-9.
24. Mathieu C, Ecochard R, Bied V, Lornage J, Czyba JC. Cumulative conception rate following intrauterine artificial insemination with husband's spermatozoa: influence of husband's age. Hum Reprod 1995; 10:1090-7.
25. Irvine DS, Aitken RJ. Predictive value of in-vitro sperm function tests in the context of an AID service. Hum Reprod 1986;1:539-45.
26. Hinney B, Wilke G, Michelmann HW. Prognostic value of an automated sperm analysis in IVF or insemination therapy. Andrologia 1993;25:195-202.
27. Horvath PM, Bohrer M, Shelden RM, Kemmann E. The relationship of sperm parameters to cycle fecundity in superovulated women undergoing intrauterine insemination. Fertil Steril 1989;52:288-94.
28. Marshburn PB, McIntire D, Carr BR, Byrd W. Spermatozoal characteristics from fresh and frozen donor semen and their correlation with fertility outcome after intrauterine insemination. Fertil Steril 1992; 58:179-86.
29. Barratt CL, Tomlinson MJ, Cooke ID. Prognostic significance of computerized motility analysis for in vivo fertility. Fertil Steril 1993; 60:520-5.
30. Bielsa MA, Andolz P, Gris JM, Martinez P, Egozcue J. Which semen parameters have a predictive value for pregnancy in infertile couples? Hum Reprod 1994;9:1887-90.
31. Aitken RJ, Best FS, Richardson DW, et al. An analysis of sperm function in cases of unexplained infertility: conventional criteria, movement characteristics, and fertilizing capacity. Fertil Steril 1982; 38:212-21.
32. Confino E, Friberg J, Dudkiewicz AB, Gleicher N. Intrauterine inseminations with washed human spermatozoa. Fertil Steril 1986; 46:55-60.
33. Campana A, Sakkas D, Stalberg A, et al. Intrauterine insemination: evaluation of the results according to the woman's age, sperm quality, total sperm count per insemination and life table analysis. Hum Reprod 1996;11:732-6.
34. Berg U, Brucker C, Berg FD. Effect of motile sperm count after swim-up on outcome of intrauterine insemination. Fertil Steril 1997;67:747-50.
35. Liu DY, Baker HW. The proportion of human sperm with poor morphology but normal intact acrosomes detected with Pisum sativum agglutinin correlates with fertilization in vitro. Fertil Steril 1988;50: 288-93.
36. Chan SY, Wang C, Chan ST, et al. Predictive value of sperm morphology and movement characteristics in the outcome of in vitro fertilization of human oocytes. J In Vitro Fert Embryo Transf 1989;6: 142-8.
37. Sukcharoen N, Keith J, Irvine DS, Aitken RJ. Predicting the fertilizing potential of human sperm suspensions in vitro: importance of sperm morphology and leukocyte contamination. Fertil Steril 1995;63:1293-300.
38. Francavilla F, Romano R, Santucci R, Poccia G. Effect of sperm morphology and motile sperm count on outcome of intrauterine insemination in oligozoospermia and/or asthenozoospermia. Fertil Steril 1990;53:892-7.
39. McGowan MP, Baker HW, Kovacs GT, Rennie G. Selection of high fertility donors for artificial insemination programmes. Clin Reprod Fertil 1983;2:269-74.