Prognostic Factors of Pregnancy after Homologous Intrauterine Insemination

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Abstract

The objective was to identify the factors influencing the outcome of homologous intrauterine insemination (IUI-H). Nine hundred and sixty-eight couples underwent 2246 IUI-H cycles. The clinical pregnancy rate (PR) per IUI-H cycle and per couple, and the miscarriage and multiple pregnancy rates were 11.8%, 27.4%, 23.3%, and 12.0%, respectively. IUI-H for combined infertility (n = 118) was 3 times less efficient than IUI performed for unexplained infertility (n = 289), OR = 0.293 (95% CI, 0.098-0.872). PRs did not differ in CC, letrozole and gonadotropin cycles, but compared with CC, Gonal-F was twice as efficient, OR = 1.994 (95% CI, 1.137-3.495). The cycles with at least 3 follicles ≥17 mm were twice as successful as the cycles with 1 follicle only, OR = 1.836 (95% CI, 1.061-3.177). The success of IUI-H increases with higher inseminated motile sperm count (IMC), from $3.6×10^6$ up to $12×10^6$, where it reaches a plateau.

Keywords: Inseminated motile sperm count; Intrauterine insemination; Recombinant gonadotropins; Types of infertility

Introduction

Intrauterine insemination (IUI) is the first-choice method in the treatment of infertility due to moderate oligoasthenoteratozoospermia, ovulatory dysfunction, surgically treated stage I or stage II endometriosis, cervical and unexplained infertility, when at least one fallopian tube is patent. Studies report variable pregnancy (PRs) and delivery (DRs) rates, and outline many IUI outcome predictors in often large retrospective series or reduced prospective studies. The type and extent of fresh sperm quality impairment, motility, and sperm morphology [1], and inseminated motile sperm count (IMC) after sperm preparation are often the most important sperm parameters that predict IUI success [2]. However, the IMC threshold level, above which IUI can be performed with acceptable PRs has not been determined yet. Other important male factors of influence are sexual abstinence before IUI, technique of processing fresh sperm [3], sperm quality as assessed by computer-assisted sperm analysis [4], abnormal hemizona assay [5], abnormal ionophore-induced acrosome reaction [6], post-wash total sperm count [7], sperm preparation time [8], the means for prepared sperm insertion into the female genital tract, time and number of inseminations [9], and immediate absolute rest after IUI [10]. Female positive predictors for successful IUI are women's age ≤38 years [11], good ovarian reserve, higher number of antral [12] and mature follicles developed [12-15], increased endometrial thickness, and good endometrial and subendometrial vascularization [16]. Female factors influencing PRs negatively are longer duration of infertility and primary infertility [11], high number of cycles performed [17], and the use of alcohol, coffee, and tea in the past [18]. The multitude of factors that have been proposed to play a role in the prediction of IUI outcome suggest that a powerful predictor has not been found yet, and that it remains difficult to predict IUI outcome. To identify the factors influencing IUI outcome, we analysed the results of homologous IUI (IUI-H) cycles performed between 2002 and 2011 at the Andrology Unit of the Division of Obstetrics and Gynecology, UMC Ljubljana. For this purpose, we observed the types of infertility, protocol of ovarian stimulation used and ovarian response, and threshold levels of inseminated motile sperm count below which IUI must be avoided or above which the IUI pregnancy outcome is no longer enhanced.

Subjects and Methods

Study populations: cycles and patients

We retrospectively analysed 2246 IUI-H cycles in 968 couples performed between 2002 and 2011 at the UMC Ljubljana (Andrology Unit, Division of Obstetrics and Gynaecology). The couples were treated for primary infertility in 676 cases (69.8%) and secondary infertility in 292 cases. Before treatment, the couples underwent an infertility assessment including two semen analyses, determination of serum follicle-stimulating hormone (FSH), hysterosalpingography or laparoscopy. IUI was proposed to couples with the following indications; 1. Female infertility: ovulation abnormality (anovulation, polycystic ovaries or diminished ovarian reserve), endometriosis with patent tubes, history of myomectomy or resection of uterine malformations, unilateral tubal obstruction and cervical pathology; 2. Moderate male infertility according to the World Health Organization (WHO) guidelines [19], with at least 1 million motile sperm after sperm preparation. 3. Combined infertility, and 4. Unexplained infertility.

The mean duration of infertility was 2.1 years (range 0.5-18 years). The mean female and male age at the first IUI attempt was 31.3 ± 3.8 years (range 22-44 years), and 34.1 ± 4.9 years (range 18-66 years), respectively, and the mean female body mass index (BMI) was 23.0 ± 4.2 kg/m² (range 15.2 - 46.4 kg/m²). Each couple underwent 1.9 ± 1.0 IUI cycles on average (range 1-8). The study protocol was approved by the institutional ethics committee.

Number of attempts	No. of cycles	Clinical pregnancies/cycles (%)	Miscarriages/all pregnancies (%)	Multiple pregnancies/all pregnancies (%)
1	968	123/968 (12.7)	34/123 (27.6)	17/123 (13.8)
2	687	81/687 (11.7)	11/81 (13.5)	7/81 (8.6)
3	360	42/360 (11.6)	11/42 (26.1)	6/42 (14.2)
4	206	17/206 (8.2)	6/17 (35.2)	2/17 (11.7)
5	18	2/18 (11.1)	0	0
6 and more	7	1/7 (14.2)	0	0
Total	2246	266 (11.8)	62 (23.3)	32 (12.0)

Table 1: Intrauterine insemination clinical pregnancy, miscarriage and multiple pregnancy rates by number of attempts for the total of 2246 cycles.

Fresh sperm quality

Diagnostic fresh sperm analysis was performed 3 to 6 months before IUI. Classical sperm characteristics (concentration, motility, normal morphology, vitality, leukocytes, and antisperm antibodies) were determined according to WHO. Sperm morphology was assessed using strict Tygerberg criteria. Sperm was considered normal, if the sperm count was $\geq 40 \times 10^6$ in the ejaculate, rapid progressive motility exceeded 25%, and normal morphology was $\geq$14%. Leukocytes and antisperm antibodies were determinedusing peroxidase method and mixed antiglobulin reaction (MAR)-test (SpermMarIgG, IgA test, FertiPro NV, Beernem, Belgium). Immunologic infertility was suspected, if there were more than 50% motile sperm with bound particles. Male infertility was diagnosed, if the semen quality did not meet the WHO criteria.

Female and male indications for IUI – types of infertility

At least one infertility factor was found present in 547 (58.5%) women: ovulatory dysfunction, American Fertility Society stage I and II endometriosis, and history of surgically corrected septate uterus and/or myomectomy in 141, 184, and 150 women, respectively. Unilateral tubal patency was observed in 34, and isolated cervical factor infertility in 13 women (Table 2). Sperm analysis before IUI was abnormal in 235 (25.1%) men. Semen abnormalities such as asthenozoospermia, teratozoospermia, oligoasthenoteratozoospermia, leucocytospermia and antisperm antibodies associated with abnormal sperm concentration, motility and morphology were observed in 131 (14.2%), 50 (5.4%), 23 (2.5%), 140 (15.2%) and 46 (5.0%) men, respectively (Table 3). In summary, 289 (30.5%) couples had unexplained infertility, in 427 (45.1%) couples the infertility was of female origin, in 111 (11.7%) of male origin, and combined in 118 (12.4%) couples.

Ovarian stimulation

The cycles were stimulated with Clomiphene Citrate (CC), Letrozole (L) and gonadotropins. In CC (Klomifen, Belupo, Koprivnica, Croatia) cycles, 50 mg of clomiphene citrate was administered twice a day from day 5 of the menstrual cycle and continued for 5 days. In the L cycles, 2.5 mg of Letrozole (Femara, Novartis Pharma, Nürnberg, Germany) was administered twice a day between days 3 and 7 of the cycle. In gonadotropin-stimulated cycles, urinary FSH (Pergonal, Merck

Serono, Darmstadt, Germany), highly purified human menopausal gonadotropin (Menopur, Ferring, Kiel, Germany), or recombinant FSH (Gonal-F, Serono, Geneva, Switzerland and Puregon, N.V. Organon, Oss, The Netherlands) were used starting on day 2 of the cycle.

Factors of female infertility	Couples n/%	Pregnancies/Pregnancy rate
No factor	388/41.4%	48/12.4%
There is a female infertility factor	547/58.5%	66/12.0%
Ovulation abnormality	141/15.0%	18/12.7%
Endometriosis	184/19.7%	20/11.6%
Uterine malformation, myoma	150/16.0%	19/12.7%
Tubal pathology	34/3.6%	3/8.8%
Uterine cervix pathology	13/1.4%	2/15.4%
Other pathologies	16/1.7%	4/25.0%

Table 2: Female infertility factors and IUI success.

Male infertility according to sperm analysis	Couples n/%	Number of pregnancies/ Pregnancy rate
Normal semen parameters	529/57.6%	75/14.2%
Abnormal semen parameters	390/42.4%	39/10.0%
Asthenozoospermia	131/14.2%	9/6.9%
Teratozoospermia	50/5.4%	2/4.0%
Oligoasthenoteratozoospermia	23/2.5%	2/8.8%
Immunologic factor	46/5.0%	2/4.3%
Leukocytospermia	140/15.2%	24/17.1%

Table 3: Male infertility factors and IUI success.

The initial dose of gonadotropin prescribed depended on the woman's hormonal profile. In the first cycle, the following medications were given: CC to 350 women, L to 122 women, Gonal-F to 177

women, Puregon to 146 women, Menopur to 118 women, Pergonal to 22 women, and combinations of CC and Gonal-F or Menopur to 33 women. A pelvic ultrasound scan was performed on day 10 in CC and L cycles, and on day 8 in gonadotropin cycles to determine the number and size of follicles, and to assess the endometrial thickness; the scan was repeated, if necessary. When at least one follicle reached ≥17 mm in diameter, ovulation was triggered using human chorionic gonadotropin (Pregnyl, N.V. Organon, The Netherlands) 5000 IU in a single dose. Insemination was performed 36 hours later. In the cycles, in which spontaneous LH surge was observed, IUI was performed the following day. Ovarian response was evaluated according to the number of follicles measuring 17 mm or more in diameter.

Sperm processing – insemination

Sperm for IUI was prepared by swim-up technique (n = 123) or density gradient centrifugation (DGC) (n = 725). After 2 to 5 days of abstinence and 2 hours before insemination, semen was collected at the laboratory. After 30 min of liquefaction, semen analysis was performed.

For swim-up, 1 mL of Sperm Preparation Medium (Origio, Målov, Denmark) was layered over 1 mL of semen and incubated for 1 hour at 37°C. The uppermost 0.5 mL contained highly motile spermatozoa.

For DGC, 1.5 mL of ejaculate were prepared by centrifugation on a density gradient (PureSperm; Nidacon International, Mölndal, Sweden), diluted in Sperm Preparation Medium. After 30 min of centrifugation at 1,200 rpm, the most concentrated phase, containing the most motile spermatozoa, was resuspended in Sperm Preparation Medium and centrifuged for 10 min at 1,400 rpm. The resulting pellet was resuspended in 0.5 mL of Sperm Preparation Medium. After a number and motility evaluation, 0.4 mL were injected into the uterine cavity using soft catheters with or without a guide (IUI cannula with guidewire or standard IUI cannula; Prodimed, Neuilly-en-Thelle, France). No luteal phase hormonal support was applied. A pregnancy test was performed 14 days after insemination if the menstruation was absent. Only clinical pregnancies with cardiac activity detected by an ultrasound were considered.

Data collection and statistical analysis

The information on infertility factors, treatment protocols, ovarian response, and sperm quality, pregnancy, multiple pregnancy, and abortion rates were obtained from patients' medical records. Clinical pregnancy, multiple pregnancy, and miscarriage rates were calculated for the whole group of 2246 cycles by the number of attempts. To avoid biases due to multiple cycles performed by some couples, only first-attempt cycles (n = 968) were taken into account in the final analysis of factors influencing the success of IUI.

Bivariate analysis (Mann-Whitney U and χ^2 tests) of first attempt H-IUI cycles was performed first, and was followed by logistic regression.

Logistic regression analysis was used to test the association between the type of infertility (categorized as unexplained infertility, female and male infertility and combined infertility), ovarian stimulation protocols (categorized as cycles stimulated by CC, gonadotropins, letrozole, and combinations), ovarian response (number of preovulatory follicles) and Inseminated Motile Sperm Count (IMC), and the occurrence of pregnancy. Data were analysed using SPSS software version 19.0 and R program (http://www.R-project.org/). Statistical significance was set at p<0.05.

Results

Two hundred and sixty-six pregnancies were achieved. There were 62 miscarriages (23.3%), 4 tubal pregnancies, and one molar pregnancy. The multiple pregnancy rate was 12.0%. The overall clinical pregnancy rate (PR) per cycle and per couple was 11.8% and 27.4%, respectively; the delivery rate (DR) per cycle was 8.8%. The proportions of singleton, twin and triplet deliveries were 87.4%, 12.6%, and 0.0%, respectively. PRs did not differ statistically among the cycles. Clinical pregnancy, miscarriage and multiple pregnancy rates for the total of 2246 cycles by the number of attempts are presented in Table 1. No fetal reduction was performed. PR in ovulatory dysfunction, stage I and II endometriosis and history of surgically corrected septate uterus and/or myomectomy was 12.7%, 11.6%, and 12.7%, respectively (Table 2). PR was lower in cases of asthenozoospermia (6.9%, p = 0.02) and teratozoospermia (4.0%, p = 0.04), compared to the couples with normal sperm parameters. H-IUI success was not influenced by isolated leukocytospermia (17.1%) (Table 3). No influence of the following parameters on PR was observed: order (p = 0.478) and duration of infertility (p = 0.192), female (p = 0.753) and partner's age (p = 0.493), smoking (p = 0.489), BMI (p = 0.159), endometrial thickness (p = 0.634), and technique of sperm processing (p = 0.286). Stimulation by CC and L was less successful than that with gonadotropins (p < 0.001). Logistic regression analysis (Table 4), used for first attempt cycles only, showed that IUI-H for combined infertility was less successful than IUI for unexplained infertility, OR = 0.293 (95% CI, 0.098-0.872).

	Pregnancy rate (%)	B	P-value	Odds ratio	95% confidence interval	
					Lower	Upper
Unexplained infertility	15.6		0.105			
Female infertility	14.1	-0.207	0.392	0.813	0.507	1.305
Male infertility	8.2	-0.707	0.139	0.493	0.193	1.258
Combined infertility	5.2	-1.229	0.027	0.293	0.098	0.872
Clomiphene citrate	10.3		0.069			
Gonal-F	20.3	0.69	0.016	1.994	1.137	3.495
Menopur	15.3	0.266	0.451	1.305	0.653	2.608
Puregon	10.3	-0.027	0.942	0.974	0.473	2.006
Letrozole	9.1	-0.544	0.29	0.58	0.212	1.591
Combinations	11.7	-0.233	0.682	0.792	0.26	2.41
1 ≥ 17 mm follicle	10.3		0.067			
2 ≥ 17 mm follicles	12	0.085	0.757	1.089	0.635	1.865
3 and more ≥ 17 mm follicles	14.9	0.607	0.03	1.836	1.061	3.177
B = regression coefficient						

Table 4: Factors influencing pregnancy rate in 968 couples undergoing first attempt of IUI-H tested by logistic regression.

Ovarian stimulation by recombinant gonadotropin Gonal-F was associated with better PRs than CC, OR = 1.994 (95% CI, 1.137-3.495). PRs were higher when three or more preovulatory follicles were present at the time of LH surge in comparison with only one follicle present, OR = 1.836 (95% CI, 1.061-3.177). The PR was positively associated with IMC. The lowest IMC with which we obtained a pregnancy was 3.6×10^6. The exact relationship between PRs and IMC was assessed using restricted cubic splines. Figure 1 shows that the success of IUI-H increased to 12 million of motile sperm, and then levelled off. Low IMC was associated with almost negligible chances of success – cycles with IMC$<5 \times 10^6$ yielded a PR of less than 2%.
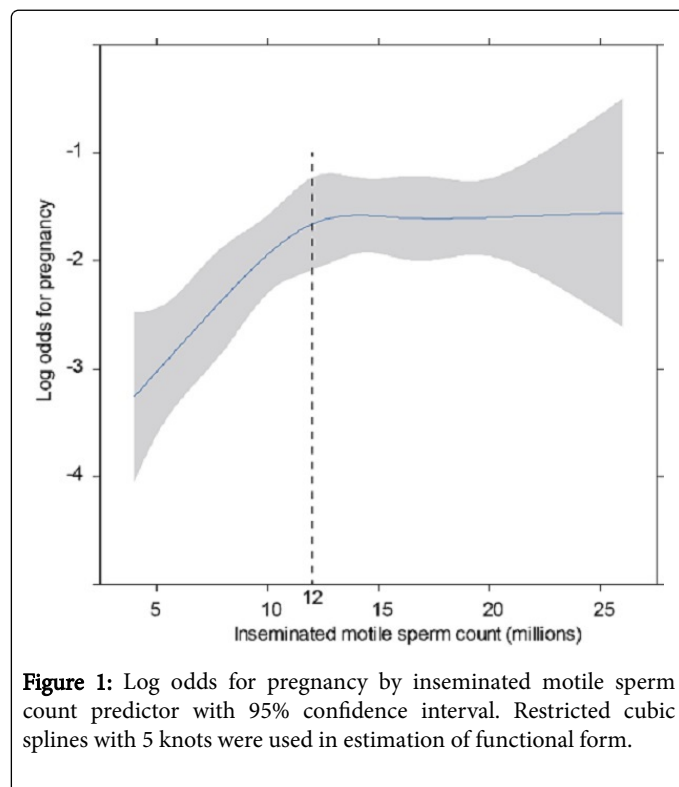

Figure 1: Log odds for pregnancy by inseminated motile sperm count predictor with 95% confidence interval. Restricted cubic splines with 5 knots were used in estimation of functional form.

No significant correlation was observed between the above tested variables and the occurrence of a miscarriage and live birth.

Discussion

The highest PRs were achieved in couples with unexplained infertility, stimulated by recombinant gonadotropins, with more than three preovulatory follicles and more than 5×10^6 motile sperm inseminated.

In the 10-year period we managed to reach an 11.8% PR and an 8.8% DR per cycle, which is comparable to the average PRs and DRs obtained in European ART centres in 2010 [20].

One of the important factors influencing pregnancy rates following IUI-H is the use of controlled ovarian stimulation (COS) protocols. The results of our study show that the increased number of preovulatory follicles influence the PR positively: patients with ≥3 follicles had twice as much chance to conceive as those developing only one follicle, which is in accordance with other reports [14]. However, the increased incidence of multiple pregnancies is the disadvantage of the COS IUI cycles. The opinion about how to face the problem of multifollicular development, and consequently multiple pregnancies

has changed over time. In 1996, Valbuena et al. [21] recommended to cancel the IUI cycle, if there were more than six preovulatory follicles present at the time of insemination. In 2002, Kaplan et al. [13] found that three to four mature follicles yielded improved cycle fecundity as compared with that in cycles with a smaller or larger number of follicles. However, in 2008 van Rumste et al. [15] stated that the presence of three or four mature follicles was associated with an increased multiple pregnancy rate following IUI without a substantial gain in the overall pregnancy rate. They concluded that ovarian stimulation for IUI should not aim for more than two follicles. If safety is the primary concern, one stimulated follicle should be the goal, whereas two follicles may be acceptable only after careful patient counselling [15]. Mild ovarian hyperstimulation has been proposed to avoid multiple pregnancy while maintaining high pregnancy rates. In order to maintain low multiple pregnancy rates in stimulated IUI cycles, the patients with four or more mature follicles might be offered an IVF cycle instead. Every decision is then individualised taking into account factors which contribute to the success or failure of the treatment, i.e. increased age and previous cycles, miscarriages. The relatively low multiple pregnancy rate (12%) we obtained is comparable to that reported in the ESHRE report for 2010 by Kupka et al. [20].

Regarding ovarian stimulation, gonadotropins did not offer superior PRs than clomiphene citrate which is not consistent with the studies reporting the advantages of gonadotropins to CC and aromatase inhibitor letrozole [21,22], but in agreement with Dankert et al. [23]. However, when gonadotropins were considered separately, better PRs were achieved with recombinant Gonal-F than with CC and letrozole. This may be explained by differences in the efficiency observed between recombinant gonadotropins vs. urinary gonadotropins [24], and recombinant gonadotropins vs. highly purified gonadotropins [25].

A close relationship between sperm quality and successful IUI has been reported in literature. However, there has been no agreement on a potential threshold above which IUI is to be proposed. Van Voorhis et al. [26] reported that IUI was efficient only if there were at least 10×10^6 motile sperm in the ejaculate. Other authors prefer to consider inseminated motile sperm count (IMC) instead; IUI used for treating male factor infertility has little chance of success when the number of motile spermatozoa inseminated is $<5 \times 10^6$ [27].

In a similar retrospective study to ours, Khalil et al. [28] did not recommend IUI if less than 5 million motile sperm were prepared. We have established that IUI-H cycles with the IMC$<5.0 \times 10^6$ have low chances to succeed.

We have observed the highest PR in women with no known infertility factor whose partners were normozoospermic. IUI for combined infertility resulted in the poorest PR and is to be avoided in these cases. This is consistent with Ahinko-Hakamaa et al. [29] who stressed the importance of the etiology of infertility that should be taken into account before planning an IUI.

Contrary to many authors [11,30], we have not found the increasing female partner's age to affect IUI outcome negatively This is likely related to the fact that in our centre female partners aged >37 years are immediately managed with IVF.

Also, we have not found a relationship between the type of infertility (primary vs. secondary) and the occurrence of pregnancy.

Neither have we found a relationship between IUI and duration of infertility, endometrial thickness and the technique of sperm preparation. As regard to swim-up and density gradient centrifugation, the differences in the number of either technique used (128 swim-up vs. 725 DGC) can be explained by the fact that after the publication of Morshredi et al. [31] in 2004 indicating that "although samples with an acceptable number of motile sperm can be processed efficiently by wash only, poor quality semen samples should be processed using DGC", we decided that sperm would be prepared exclusively by DGC. The fact that the threshold for IUI we determined is relatively higher than that proposed by Dinelli et al. [11] and Cao et al. [32] is probably due to the fact that we did not include the couples with extremely poor sperm in the study; those couples being treated by ICSI. The big limitation of our study is its retrospective nature.

During the study period the techniques of sperm preparation changed, and the ovarian stimulation protocols were not equally represented (350 patients were treated with clomiphene, 463 with gonadotropins, but only 122 took letrozole). Moreover, women older than 37 years and men with very poor sperm quality were not offered IUI as explained above.

Nonetheless, beside female partner's age, there are three factors of importance that predict a successful IUI outcome: unexplained infertility, good ovarian response, and $\geq 5.10^6$ motile spermatozoa inseminated.

Authors' contributions

BZ and EVK conceived and designed the retrospective study.

BZ drafted the paper and prepared the final version of the manuscript.

IV performed the statistical analyses.

MK supervised classical sperm analysis and prepared sperm for IUI.

Competing interests

The authors declare that they have no competing interests.

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