Scientific
Research
Publishing

Intrauterine Insemination—Our Results between the Years 2008-2012

Vilma Lánská, Blanka Koubková, Jitka Řezáčová, Michal Krčmář, Pavel Darebný, Lucie Melicharová, Kateřina Dohnalová, Alena Vaverková, Jaroslav Feyereisl

Institute for the Care of Mother and Child, Prague, Czech Republic
Email: vilma.lanska@centrum.cz

Abstract

The purpose of this retrospective study was to determine whether or not there was a significant relationship between women's age as a cause of sterility and pregnancy after IUI. Furthermore, we evaluated the effect of stimulation of antiestrogens and time of hCG administration on the success of IUI in relation to the age of women. During the period between 2008 and 2012, we performed a total number of 793 IUI. Patients were prepared for IUI in the natural cycle and stimulation with antiestrogens (clomiphene citrate-CC). Ovulation was induced by hCG (Ovitrelle) 40 hours before IUI or immediately after the procedure. Sperm was processed through density gradients. The average success rate of IUI was 10.2% of pregnant women per cycle. Significantly the highest number of pregnant women 16.3% was women with a diagnosis of anovulation. Significantly the lowest success rate of IUI was at the immunological cause of infertility and endometriosis. There was no evidence of age dependence for women on the success of IUI. Stimulation of CC did not significantly increase the chance of becoming pregnant. There was also no statistically significant difference in hCG before and after IUI pregnancy success. The most important group of women for whom IUI is a suitable form of assisted reproduction consists of patients of 35 years old with anovulation cause of sterility. Those patients with an immunological cause of infertility and endometriosis have significantly lower chances of conceiving after IUI and it is preferable for them to choose other techniques of assisted reproduction and embryo transfer.

Keywords

1. Introduction

Intrauterine insemination (IUI) is now regarded as the simplest form of assisted reproduction and still used reg-

ularly in the treatment of fertility problems. The principle of the method is the injection of sperm by catheter through the cervix into the uterine cavity during the ovulation period. The chance of pregnancy after this technique is around 7% in a natural ovulation cycle and 10% to 13% after stimulation of oocyte growth by clomiphene citrate (CC) and induction of ovulation using human chorionic gonadotrophine (hCG).

When using the follicle stimulating hormone (FSH), pregnancy rates increase to 14% - 18%, but its administration also significantly increases the risk of ovarian hyperstimulation syndrome (OHSS) and multiple pregnancies [1]. The European Society of Human Reproduction and Embryology (ESHRE) Capri Workshop Group reported that a pregnancy rate of 12.4% in 98,388 cycles of IUI performed in 2004. The incidence of multiple births with IUI was 11.8%, suggesting that most cycles were combined with controlled ovarian hyperstimulation [2]. The price of FSH, increased the risk of complications after stimulation, the higher likelihood of multiple pregnancies, and the related risk of miscarriage and premature births represent the reasons why this type of hormonal stimulation before IUI is not used in our department.

The etiology of infertility has an important role in prognosis of IUI cycles [3] [4]. Indications for IUI: 1) light disorder of male fertility when laboratory processed ejaculate increases the concentration of motile sperm that is injected in nutrient solution through an insemination catheter via cervix into the uterine cavity. When azoospermia of partner occurs, then it is possible to use donor sperm; 2) the immunological cause of infertility in which sperm cannot penetrate the uterine neck to the upper parts of the female genitalia; 3) anovulation syndrome when using the CC (or FSH) that induces the growth and maturation of follicles in the ovary and using hCG for timing of ovulation; 4) unclear cause of fertility problems when there is also possible accurate timing of insemination by using CC (or FSH) and hCG.

Conditions for intrauterine insemination: 1) at least one Fallopian tube feasible; 2) at least one million motile sperm after laboratory preparation; 3) hormonal stimulation with minimal risk of multiple pregnancies.

The aim of this retrospective study was to determine whether there was a significant relationship between women's age as a cause of sterility and pregnancy after IUI. Furthermore, we evaluated the effect of stimulation of antiestrogens and timing of hCG administration on the success of IUI in relation to age of women.

2. Materials and Methods

2.1. Study Participants

This study retrospectively considered the consecutive IUI carried out at the Centre of Assisted Reproduction of the Institute for the Care of Mother and Child, Prague, Czech Republic. The study was approved by the Ethical Committee of the Institute for the Care of Mother and Child.

During the period 2008-2012, a total number of 793 IUI were performed, of which 687 (86.6%) were from husbands (Artificial Insemination Husband—AIH) and 106 (13.4%) from donors (Artificial Insemination Donor —AID). The mean age of women treated with this method of assisted reproduction was 32.8 years old (22 - 45 years old), mean age of men was 34.8 years old (23 - 59 years old). The causes of infertility have been divided into: male factor, anovulation syndrome, idiopathic and immunological factors including endometriosis.

2.2. IUI Procedures

Patients were prepared for IUI: 1) in the natural cycle (*i.e.* without hormonal stimulation) and 2) the stimulation with anti-estrogens (clomiphene citrate-Clostilbegyt, EGIS Pharmaceuticals PLC, Hungary, 50 mg, 2 tablets daily from 5th to 9th day of the period). The first ultrasound examination (folliculometry and endometrial thickness) was performed on the 10th-11th day of the period. Further ultrasonographic check-ups were performed every other day depending on the size of follicles. When follicle size grew up to the limit of 15 - 18 mm, hCG (1 amp Ovitrelle sc., Merck Serono S. P. A., Italy) was applied at 7 PM. 40 hours later, after application of hCG, the IUI procedure was performed. When follicle size was larger than 18 mm, IUI was performed the next day with hCG application immediately after the procedure. Only IUI with maximum of three follicles was performed.

All procedures were performed on an outpatient basis. In the gynecological position, Cusco's vaginal speculum was inserted, uterine cervix was fixed and with the use of a Wallace Artificial Insemination Catheter (Smiths Medical International, UK) cervical canal was inserted into the uterine cavity and 1 ml of nutrient solution with sperm was injected. After removal of all tools the patient remained in a horizontal position for 15 mi-

nutes. After the procedure a return to normal activities was allowed and regime restrictions were not necessary.

An HCG assay was done 18 days after the IUI and if positive we made the first ultrasound diagnosis of pregnancy within the next 10 days. After the date of IUI patients began gestagenic support (Utrogestan, Besins Manufacturing Belgium SA, Belgium, 2-0-2 vaginally) until we were able to determine the results of a pregnancy test. Upon positive findings, they continued until the 8th week of pregnancy.

2.3. Sperm Preparation

Samples of sperm for AIH were obtained by masturbation 60 - 90 minutes before the procedure. After 20 minutes of the sample liquefaction native spermiogram was evaluated for ejaculate volume, sperm concentration, total motility and morphology of the predominant spermatic movement in a Makler Counting Chamber (Sefi-Medical Instruments, Israel).

Spermatozoa were separated from seminal plasma by centrifugation through a density gradient (80% Lower Phase Gradient, 40% Lower Phase Gradient, Sage Media, USA). The sample was centrifuged for 20 minutes at 1600 per minute $417 \times$ g. After removing of the supernatant, the pellet was resuspended in 3 ml Quinn's Sperm Washing Medium (Sage Media, USA) and the sample was centrifuged for 5 minutes at 1400 per minute $319 \times$ g.

After subsequent removal of the supernatant and resuspension in 1 ml Quinn's Sperm Washing Medium, final value of sperm concentration was subtracted as well as their total motility, predominant motion and sperm morphology. A suspension of sperm in Sperm Washing Medium was aspirated into a 1 ml syringe (Luer BD Plastipak BD Falcon, USA) and stored in a thermostat.

For AID frozen donors semen was used in Sperm Freezing Medium (Origio Medicult A/S, DK) at a ratio of 1:1 using a freezing apparatus Kryo 360 - 1.7 (Planer, UK). The sample pellet (CBS High Security Sperm Straw 0.5 ml, Cryo Bio System, France) was thawed for 10 minutes at room temperature and then processed identically to freshly collected samples of semen. Spermatozoa were prepared by procedure recommended by manufacturer Sage Media.

2.4. Statistics

Data were subjected to statistical analysis. The results were evaluated by Pearson chi-square analysis (**Tables 4-6**) and Fisher Exact Test (**Table 7**). A p value of less than 0.05 was considered significant.

3. Results

During the years 2008-2012 a total number of 793 IUI procedures were performed at our workplace. The average age of women treated with this method of assisted reproduction was 32.8 year old, mean age 34.8 year old for men. **Table 1** shows the frequency of procedures performed, depending on the age of patients, age to 35 years old, 35 - 40 years old and women 40 - 45 years old. The largest group consisted of women up to 35 years old. The men were divided into groups under 30 years old, 30 - 40 years old and above 40 years old, whereas the largest group consisted of men 30 to 40 years old (**Table 2**).

Of the total number of IUI performed we achieved 81 clinical pregnancies (10.2%), in 79 cases a single pregnancy and in 2 cases twin pregnancy. The study recorded 57 patients were held for births, 10 cases of miscarriages and two ectopic pregnancies. In 12 cases it could not be determined whether women had given birth or miscarried.

The file was then evaluated according to the quality of men's sperm analysis at IUI. The largest group consisted of men with normozoospermia (659 cases, 83.1%), followed by group oligoasthenozoospermia (105 cases, 13.2%) and least frequent was a group of men with a final evaluation of teratozoospermia (29 cases, 3.7%). 67 pregnancies were established after insemination with samples of normozoospermia, 11 pregnant women made pregnant after IUI with samples of oligoasthenozoospermia and finally 3 pregnancies following IUI with samples of teratozoospermia.

In our study, we focused on comparing IUI success depending on the causes of fertility. The causes of sterility were divided into male factor, anovulation factor and idiopathic causes including immunological causes and endometriosis. Distribution of causes of infertility in women depending on age can be seen from **Table 3**. Among women up to 35 years old and 35 - 40 years old, the most inseminations indicated were for idiopathic sterility and in women 40 - 45 years old were due to anovulation respectively. However, the difference is not statistically significant. Overall, the most IUI was performed for idiopathic sterility.

Table 1. IUI frequency in women by age.

Women's Age Group		
Years Old	Frequency	%
age to 35	562	70.9
35 - 40	181	20.8
40 - 45	50	6.3
Total	**793**	**100**

Table 2. IUI frequency in men by age.

Men's Age Group		
Years Old	Frequency	%
age to 30	179	22.6
30 - 40	517	65.2
40 - 45	97	12.2
Total	**793**	**100**

Table 3. Age of the patients and the cause of sterility.

	Cause of Sterility (Frequency)			
Age (Years Old)	Male Factor	Idiopathic	Imunologic + Endometriosis	Anovulation
to 35	176	215	78	94
35 - 40	60	74	26	21
40 - 45	8	19	3	20
Total	**244**	**308**	**107**	**135**

Furthermore, **Table 4** shows the number of pregnancies after IUI depending on the cause of infertility. An average success rate of IUI was 10.2% for pregnant women in the cycle. Significantly, the highest number of pregnant women 16.3% was among women with anovulation. Significantly, the lowest success of IUI was accounted by the immunological cause of infertility and endometriosis.

We also watched the number of pregnant women as well after IUI depending on their age. As can be seen from **Table 5**, the highest number of pregnant women was in the group up to age of 35, but the result is not statistically significant.

Patients were prepared for IUI in a native cycle without hormonal stimulation or stimulation with antiestrogens-clomiphene citrate (CC). We examined the effect of administration of CC on the success of IUI by the age of women. **Table 6** shows that for young women up to age 35 (in the case that women have ovulatory cycle), the use of CC does not increase the pregnancy rate. The success of IUI without the administration of CC was higher, but the difference was not statistically significant. For women over 35 years the administration of antiestrogens is desirable, especially in women older than 40 years, where there are higher chances of getting pregnant after administration of CC.

Another endpoint was the time of hCG administration—Ovitrelle (**Table 7**). If ultrasound showed follicle size 15 - 18 mm, Ovitrelle was applied at 7 PM. 40 hours after the application, IUI was performed. When follicle size became larger than 18 mm, IUI was performed the following day with Ovitrelle applications immediately after the procedure.

In CC stimulated cycles a higher success rate was recorded, when applying Ovitrelle 40 hours prior to the procedure, regardless of age. When applying Ovitrelle after the procedure, the chances of pregnancy reduced in proportion to age. The differences after statistical evaluation are not significant.

Without CC stimulation we observed the highest pregnancy rate after IUI in a group of women up to 35 years

Table 4. Dependence of pregnancy rate on sterility cause.

Cause of Sterility	Pregnancy Rate (%)
male factor	9.9[a]
idiopathic	10.1[a]
imunologic + endometriosis	3.7[b]
anovulation	16.3[c]
Total	**10.2**

The different superscripts [a-c] denote statistically significant differences in the pregnancy rate ($p < 0.05$).

Table 5. Dependence of pregnancy rate on women's age.

Women's Age (Years Old)	Pregnancy Rate (%)
to 35	11.2[a]
35 - 40	7.7[a]
40 - 45	8.0[a]
Total	**10.2**

[a]No statistically significant differences in the percentage of pregnant.

Table 6. Influence of CC administration on IUI pregnancy rate (pr) depending on age.

	Clomiphene Citrate	
	with	without
Women's Age (Years Old)	pr (%)	pr (%)
to 35	10.8[a]	14.5[a]
35 - 40	9.6[a]	5.2[a]
40 - 45	9.9[a]	0.0[a]
Total	**9.9**	**10.8**

[a]No statistically significant differences in the percentage of pregnancy rate.

Table 7. Pregnancy rate (pr) depending on age, administration of CC and time of administration of Ovitrelle.

Women's Age (Years Old)	pr (%) Clomiphene Citrate +				pr (%) Clomiphene Citrate −			
	Freq.	Ovitrelle Before	Freq.	Ovitrelle After	Freq.	Ovitrelle Before	Freq.	Ovitrelle After
to 35	348	10.1[a]	56	8.9[a]	137	14.6[a]	22	13.6[a]
35 - 40	89	10.1[a]	15	6.7[a]	10	5.8[a]	8	0.0[a]
40 - 45	35	11.4[a]	2	0.0[a]	69	0.0[a]	3	0.0[a]
Total	**472**	**10.2**	**73**	**8.2**	**216**	**11.1**	**33**	**9.1**

[a]No statistically significant differences in the percentage of pregnancy rate.

old after application Ovitrelle 40 hours prior to the procedure. A statistically insignificantly lower proportion of pregnant women were in the same age group after application Ovitrelle immediately after procedure. Low success rates of IUI without CC stimulation occurred in women in groups of 35 - 40 years old and 40 - 45 years old regardless of the time of Ovitrelle administration. And the low frequency of IUI in these age groups has not demonstrated a statistically significant association between pregnancy and the time of Ovitrelle administration.

4. Discussion

In our study, the woman's age did not significantly affect pregnancy rates. Likewise in study of Soria *et al*. [5] age was not a strong predictor of success. They described the pregnancy rates per cycle for age brackets below 30 years old, 30 to 35 years old, and 35 to 40 years old, as 11.4%, 10.6%, and 9.6%, respectively. In a study by Araújo *et al*. [6] there was no significant difference in age (both men and women) in both groups (pregnant and non-pregnant). On the contrary, Demir *et al*. [7] considered age as an important factor in achieving pregnancy. They assessed 212 infertile couples and obtained a pregnancy rate of 15.8% per cycle. The pregnancy rate was the highest in IUI cycles when woman were <25 years old.

IUI is a widely used resource for the treatment of infertile couples, with greater chances for success when the IUI procedure is properly selected. The results of our retrospective analysis clearly show that the largest group of women for whom IUI is suitable occurs in patients 35 years old with anovulation cause of sterility. Average success rate of IUI was 10.2% of pregnant women in the cycle, with a significantly high number of pregnant per cycle 16.3% were women with anovulation.

In a study by Soria *et al*. [5] the pregnancy rate was 10.2% per cycle and the pregnancy rate per cycle for patients with anovulation was 13.3%. A statistically significant lowest success rate of IUI occurred in the immunological cause of infertility and endometriosis. Soria *et al*. [5] also described the lowest pregnancy rate 6.4% for cases with endometriosis. Similar results were obtained by Vlahos *et al*. [8] who reported a pregnancy rate per cycle of 19.1% for cases with anovulation, compared to 9.1% for cases with endometriosis.

Our results confirm the significant relationship between this etiology and IUI outcomes. Our results also show that for the young women of 35 years old with good ovulatory cycle, CC support does not significantly increase the pregnancy rate. In patients over 35 years old in the case of anovulation, administration of CC and Ovitrelle is required.

Clomiphene citrate is able to stimulate ovulation by competing with estrogen for binding to the hypothalamic estrogen receptors. By effectively diminishing the pool of available estrogen receptors within the hypothalamus, the negative feedback signal induced by estrogen is blocked, which in turn alters pulsatile GnRH secretion. This altered GnRH secretion then enhances gonadotropin release from the pituitary. Increased gonadotropin release ultimately drives folliculogenesis at the level of the ovary [9].

In a compilation of seven published studies looking at CC and ovulation, Homburg [10] reported an ovulation rate of 73% with a pregnancy rate of 36%. For women over 35 years old, administration of antiestrogens is highly appropriate, especially in women older than 40 years old, where the chances of getting pregnant after use of CC are higher.

Gomez *et al*. [11] evaluated the influence of ovarian stimulation on the success of IUI. In 433 cycles insemination was performed during a natural cycle. 4020 cycles were stimulated with recombinant FSH, 596 cycles with clomiphene citrate, 194 with urinary FSH, 103 with HMG. The pregnancy rates range from 7.4% in the clomiphene citrate group to 14.4% in the urinary FSH group. Clomiphene stimulation had the significantly lowest pregnancy rate. The other types of stimulation did not differ significantly from each other concerning the pregnancy rate. Patients under 39 years old did not profit from any ovarian stimulation. In 40+ patients, pregnancy rates were higher, if any stimulation had been performed.

Ovitrelle, administered 40 hours prior to IUI with induced ovulation, causes increasing pregnancy rates in comparison to its application immediately after procedure. Due to the low frequency of IUI with Ovitrelle, applications after the procedure have not shown a statistically significant association of pregnancy and the timing of Ovitrelle administration. The results of the study by Cantineau *et al*. [12] also showed no significant differences between various time intervals from hCG injection to IUI on pregnancy rates.

5. Conclusions

IUI belongs to the first-line treatments of fertility disorders in the indicated group of patients. It is a simple, inexpensive, non-invasive and secure method. Using clomiphene citrate leads to relatively low pregnancy rates, but women are not compromised by ovarian hyperstimulation syndrome or multiple pregnancies, as with the use of gonadotropins (FSH). The use of progestogens is recommended to support pregnancy after IUI [13].

The most important group of women for whom IUI is suitable are those patients at the age of 35 with anovulation cause of sterility, where the administration of CC and Ovitrelle is required for induction of ovulation. For women less than 35 years old and with another cause of infertility rather than anovulation, CC administration

before IUI fails to increase the chance of conception. Conversely, in women older than 35 years old use of CC before IUI is important.

For patients with immunological cause of infertility and endometriosis with respect to a significantly low chance of conceiving after IUI, it is preferable to choose other techniques of assisted reproduction and embryo transfer.

Acknowledgements

References

[1] Ghesquiere, S., Castelain, E., Spiessens, C., Meuleman, C. and D'Hooghe, T. (2007) Relationship between Follicle Number and (Multiple) Live Birth Rate after Controlled Ovarian Hyperstimulation and Intrauterine Insemination. *American Journal of Obstetrics and Gynecology*, **197**, 589.e1-589.e5.

[2] The ESHRE Capri Workshop Group (2009) Intrauterine Insemination. *Human Reproductive Update*, **15**, 265-277.

[3] Ahinko-Hakamaa, K., Huhtala, H. and Tinkanen, H. (2007) Success in Intrauterine Insemination: The Role of Etiology. *Acta Obstetricia et Gynecologica Scandinavica*, **86**, 855-860. http://dx.doi.org/10.1080/00016340701416895

[4] Ashrafi, M., Rashidi, M., Ghasemi, A., Arabipoor, A., Daghighi, S., Pourasghari, P. and Zolfaghari, Z. (2013) The Role of Infertility Etiology in Success Rate of Intrauterine Insemination Cycles: An Evaluation of Predictive Factors for Pregnancy Rate. *International Journal of Fertility and Sterility*, **7**, 100-107.

[5] Soria, M., Pradillo, G., García, J., Ramón, P., Castillo, A., Jordana, C. and Paricio, P. (2012) Pregnancy Predictors after Intrauterine Insemination: Analysis of 3012 Cycles in 1201 Couples. *Journal of Reproduction and Infertility*, **13**, 158-166.

[6] Araújo, L.F., Araújo, F.E., Fácio, C.L., Bossoni, M.C., Machado-Paula, L.A., Corrente, J.E., Cavagna, M., Matheus, P.C. and Pontes, A. (2013) Efficacy of Sperm Motility after Processing and Incubation to Predict Pregnancy after Intrauterine Insemination in Normospermic Individuals. *Reproductive Biology and Endocrinology*, **11**, 101. http://dx.doi.org/10.1186/1477-7827-11-101

[7] Demir, B., Dilbaz, B., Cinar, O., Karadag, B., Tasci, Y., Kocak, M., Dilbaz, S. and Goktolga, U. (2011) Factors Affecting Pregnancy Outcome of Intrauterine Insemination Cycles in Couples with Favourable Female Characteristics. *Journal of Obstetrics & Gynaecology*, **31**, 420-423. http://dx.doi.org/10.3109/01443615.2011.569780

[8] Vlahos, N.F., Coker, L., Lawler, C., Zhao, Y., Bankowski, B. and Wallach, E.E. (2005) Women with Ovulatory Dysfunction Undergoing Ovarian Stimulation with Clomiphene Citrate for Intrauterine Insemination May Benefit from Administration of Human Chorionic Gonadotropin. *Fertility and Sterility*, **83**, 1510-1516. http://dx.doi.org/10.1016/j.fertnstert.2004.11.049

[9] Clark, J.H. and Mardaverich, B.M. (1982) The Agonist-Antagonist Properties of Clomiphene: A Review. *Pharmacology & Therapeutics*, **15**, 467-519. http://dx.doi.org/10.1016/0163-7258(81)90055-3

[10] Homburg, R. (2005) Clomiphene Citrate-End of an Era? A Mini-Review. *Human Reproduction*, **20**, 2043-2051. http://dx.doi.org/10.1093/humrep/dei042

[11] Gomez, R., Schorsch, M., Steetskamp, J., Hahn, T., Heidner, K., Seufert, R. and Skala, C.E. (2014) The Effect of Ovarian Stimulation on the Outcome of Intrauterine Insemination. *Archives of Gynecology and Obstetrics*, **289**, 181-185. http://dx.doi.org/10.1007/s00404-013-2952-3

[12] Cantineau, A.E.P., Janssen, M.J., Cohlen, B.J. and Allersma, T. (2014) Synchronised Approach for Intrauterine Insemination in Subfertile Couples. *Cochrane Database of Systematic Reviews*, **12**, Article No: CD006942. http://dx.doi.org/10.1002/14651858.CD006942.pub3

[13] Agha-Hosseini, M., Rahmani, M., Alleyassin, A., Safdarian, L. and Sarvi, F. (2012) The Effect of Progesterone Supplementation on pregnancy Rates in Controlled Ovarian Stimulation and Intrauterine Insemination Cycles: A Randomized Prospective Trial. *European Journal of Obstetrics Gynecology and Reproductive Biology*, **165**, 249-253. http://dx.doi.org/10.1016/j.ejogrb.2012.08.007