

The efficiency of artificial insemination by husband sperm in infertile couples according to the revised WHO 2010 criteria

Efektywność inseminacji nasieniem męża u niepłodnych par w odniesieniu do nowych kryteriów WHO z 2010 roku

Mateusz Mikołajczyk, Tomasz Goździewicz, Jana Skrzypczak

Division of Reproduction, Poznan University of Medical Sciences, Poland

Abstract

Objectives: The object of our study was to assess the efficacy, measured as achievement of pregnancy, of artificial insemination with husband sperm in couples that fulfilled the WHO criteria for infertility.

Material and methods: We have identified 120 patients that were diagnosed with infertility, defined as at least one year of unprotected intercourse without achieving pregnancy. After 2 year follow up the study group comprised 96 women and their partners. All couples had normal outcome of all standard infertility test, except for some with decline in sperm parameters that allowed the husband sperm to be used for fertilization in the IUI procedure according to the 2010 WHO guidelines. After IUI procedure patients were followed either by contact with their physicians, mail questionnaire, or by identification of their national ID number in computerized database of our hospital.

Results: A total of 32 patients got pregnant (33%). Of those that achieved pregnancy during the two year follow up period, fifteen (46.9%) had done so as a result of AIH, another 15 as a result of spontaneous conception, and two as a result of IVF (6.2%). The mean number of AIH procedures in a group of women that did achieve pregnancy was 3.56 (median 3.0), and was statistically higher than the number of AIH in those patients who have failed to achieve pregnancy (mean 2.54; median 2.0; $p=0.009$).

Conclusions: Our study seems to support the new criteria for assessment of sperm parameters. Judging the sperm according to the new, lessened criteria, did produce comparable pregnancy rates as with historical cohorts based on old criteria.

Key words: **artificial insemination homologous / semen analysis / pregnancy rate /**

Corresponding author:

Mateusz Mikołajczyk
Division of Reproduction, Poznan University of Medical Sciences,
Polna 33 St, 60-535 Poznań, Poland
Tel. 0048616599302
E-mail: matmik@gmail.com

Otrzymano: 19.03.2013
Zaakceptowano do druku: 10.09.2013

Mateusz Mikołajczyk, et al. *The efficiency of artificial insemination by husband sperm in infertile couples according to the revised WHO 2010 criteria.*

Streszczenie

Cel pracy: Celem pracy była ocena efektywności inseminacji wewnątrzmacicznej nasieniem męża wyrażona jako uzyskane ciąże, w nawiązaniu do kryteriów niepłodności WHO.

Materiał i metody: Badaniu poddano 120 pacjentek i ich partnerów ze stwierdzoną niepłodnością, definiowaną jako niemożność zajścia w ciążę przez rok niezabezpieczonego współżycia. Po dwuletnim postępowaniu diagnostycznym do grupy badanej ostatecznie zakwalifikowano 96 par. U wszystkich par wykonano standardowe badania diagnostyczne niepłodności, w których stwierdzono tylko nieprawidłowe wyniki nasienia partnera kwalifikujące się do inseminacji wewnątrzmacicznej wg kryteriów WHO z 2010 roku. Losy pacjentek były śledzone poprzez wysłanie formularza, rozmowy z lekarzem prowadzącym lub poprzez szpitalną bazę danych.

Wyniki: Trzydzieści dwie pacjentki zaszły w ciążę (33%): 15 (46,9%) w wyniku AIH, kolejne 15 poprzez spontaniczne zapłodnienie, a dwie w wyniku procedury IVF (6,2%). Średnia liczba procedur AIH w grupie kobiet, które zaszły w ciążę wynosiła 3,56 (mediana 3,0) i była istotnie statystycznie wyższa, niż w grupie pacjentek, u których nie uzyskano ciąży (średnia 2,54, mediana 2,0; $p=0,009$).

Wnioski: Wyniki naszych badań potwierdzają zasadność nowych kryteriów parametrów nasienia. Ocena nasienia w nawiązaniu do nowych, mniej restrykcyjnych kryteriów, wiąże się z podobnym odsetkiem uzyskanych ciąż jak w przypadku starej klasyfikacji parametrów nasienia.

Słowa kluczowe: **inseminacja nasieniem męża / analiza nasienia / odsetek ciąż /**

Introduction

Infertility is defined by the WHO as lack of pregnancy after a year of unprotected intercourse. Infertility is also considered a disease that warrants proper diagnosis and treatment. It is estimated that this problem affects some 10% of couples trying to conceive [1].

There are numerous reasons for infertility, including causes arising from both male and female. Well established causes of infertility are lack of ovulation, blocked Fallopian tubes, poor sperm parameters. As far as the normal sperm parameters are concerned, there have been three changes in guidelines regarding what is supposed to be considered normal reference values. The most recent reference were published in 2010: volume 1.5 (1.4-1.7) mL, sperm concentration 15 (12-16) million/mL, total sperm number 39 (33-46) million/ejaculate, total motility 40 (38-42)%, progressive motility 32 (31-34)%, normal morphology 4 (3-4)% and vitality 58 (55-63)% [2]. With each guidelines, the reference values for normal sperm parameters were lowered, reflecting overall decline in sperm quality, lessons learned from monitoring of patients with so called "subfertility" and better selection criteria for reference values. With modern assisted reproductive technologies, even complete azoospermia or lack of spontaneous ovulation with bilateral blocked tubes does not pose a significant challenge, provided that there is access to modern IVF technologies, that is readily available for every couple requiring such therapy. However this approach, because of associated costs, is reserved for clearly guideline indications.

There is however also so called idiopathic infertility, where the couple cannot get pregnant despite all the test in normal range, or where we see only slight decline in sperm parameters. For these couples the therapy remains empiric, consisting of intrauterine insemination by husband sperm with/ without concomitant ovulation induction [3].

The object of our study was to assess the efficacy of the 2010 WHO sperm assessment criteria, measured as achievement of pregnancy, during treatment with intrauterine inseminations by husband sperm who were treated in Division of Reproduction of University of Medical Sciences in Poznan, Poland.

Material and methods

We have retrospectively identified 120 patients who were hospitalized in the Division of Reproduction Poznan University of Medical Sciences in the 2005-2011 year and were diagnosed with infertility, defined as at least one year of unprotected intercourse without achieving pregnancy- and fulfilled the recent 2010 WHO criteria for poor sperm parameters. Men, that had been initially considered to have poor semen quality according to previous WHO guidelines, but have not met the new criteria were excluded from the study. All couples had a standard panel of test performed, including: sperm analysis, ovulation tracking, patency of Fallopian tubes by either HSG (hysterosalpingography) or SIS saline infusion sonography. All patients had also full panel of hormonal studies, and laparoscopy to rule out pelvic disease like endometriosis or pelvic inflammatory disease. Couples in the study group had normal outcome of all test, except for decline in sperm parameters that allowed the husband sperm to be used for fertilization in the IUI procedure according to WHO guidelines. Only those patients that fulfilled recent 2010 WHO criteria classification were included in the analysis. The study group comprised 96 out of initial of 120 couples. In the excluded 24 couples we did not find sufficient data to include them in the final analysis.

The procedure for IUI was as follows: patients were admitted to the hospital, while on ovulation tracking, when the dominant follicle was at least 18 mm. Some patients, at their leading physician discretion, had ovulation induction with clomiphene

Mateusz Mikolajczyk, et al. *The efficiency of artificial insemination by husband sperm in infertile couples according to the revised WHO 2010 criteria.*

citrate (50-150 mg/daily from 5th-9th day of the cycle). Upon admission the patient was examined, transvaginal ultrasound was performed to assess the current ovulation status (Philips HDI 4000 with 6.5 MHz transvaginal ultrasound probe). When the dominant follicle was more than 19 mm, the husband was sent to sperm preparation facility, where after donation of sperm sample into a sterile container, the sperm was prepared with density gradient centrifugation procedure. The final solution containing sperm and some other fluid was assessed manually according to 2010 criteria by one experienced technician from

Andrology Laboratory with EQA(European Quality Assurance) certification, and transferred to Division of Reproduction. There, a soft insemination catheter from Balton was used to inject the sperm to the uterine cavity. The patients either stayed at the hospital to confirm the ovulation and formation of corpus luteum by ultrasound, or were advised to report to their leading physician for ovulation confirmation. Patients also received 20 mg of dydrogesterone or 100 mg of progesterone as a luteal phase support.

Patients were followed either by contact with their physicians, mail questionnaire, or by identification of their national ID number in computerized database of our hospital. Only patients that had full two years of follow up, or had achieved pregnancy prior to the two years period, were included in the final study group.

We used Statistica v. 10 STATSOFT software to statistical analysis. The Shapiro-Wilk test was used to analyze the character of distribution. Mann-Whitney, Student's t-test and Fisher two-sided tests were used to evaluate differences between groups. Statistical significance was accepted as being $p < 0.05$.

Results

We have identified 96 couples that met the inclusion criteria of the study. The mean age of a woman was 31.2 years old (range 23-42). There was no difference in age between women that got pregnant (mean 31.9 years of age; 24-40) and those who did not get pregnant (mean 30.9; range 23-42). The mean duration of infertility was 2.3 (1-5) years. The results of semen analysis revealed 16 patients with sperm parameters within normal range, and 80 with dysfunction – mainly with lower motility and concentration (Table I). Overall there were 277 artificial insemination with husband sperm performed in the whole group (2.9 per patient, range 1-10 AIH procedures per patient). There were also 176 concomitant ovulation inductions (mean 1.8; range 0-8) in 80 patients (83%). There were mild ovarian stimulation protocol used: monofollicular induction with clomiphene citrate (CC) 50-150 mg from 5th to 9th day of cycle. Only 11 of 80 patients (13%) required higher doses of CC (100 mg). The mean number of ovulation induction was higher in patients that achieved pregnancy (mean 2.3; 0-8; total 73) than in those who have failed to achieve pregnancy (mean 1.6;0-7; total 103), but the difference was not statistically significant $p=0.053$.

A total of 32 patients got pregnant (33%). Of those that achieved pregnancy during the two year follow up period, fifteen (46.9%) had done so as a result of AIH, another 15 as a result of spontaneous conception, and two as a result of IVF (6.2%).

The mean number of AIH procedures in a group of women that did achieve pregnancy was 3.56 (median 3.0), and was statistically higher than the number of AIH in those patients

Table I. Characteristics of 80 patients with sperm parameters dysfunction.

| Parameter | Mean ± SD |
|--------------------|------------|
| Morphology | 14% ± 11 |
| Total motility | 22% ± 15 |
| Total sperm number | 11 mln ± 8 |

who have failed to achieve pregnancy (mean 2.54; median 2.0; $p=0.009$). In the pregnancy group there were 80% (n=12) of abnormal sperm parameters in patients that achieved pregnancy as a result of AIH, one patient in the group of IVF had abnormal semen analysis, and all the patients (n=15) that got pregnant through spontaneous conception had normal semen analysis results. For one AIH procedure there was a 28.8% success in achieving pregnancy in all patients, while in the AIH group the pregnancy rate was 13.4%.

Of all the pregnancies 56% (n=18) ended in spontaneous delivery at term, while 25% (n=8) ended with term cesarean section. There are 12.5%(n=4) of ongoing pregnancies, while the pregnancy loss (defined as loss <20th week gestation) was 6.5% (n=2). There were 68.75% (n=22) of term deliveries, and 12.5% (n=4) of pregnancies ended with premature delivery-all occurred above 34th week of gestation. All premature infants survived. We also noted 9.35% twin pregnancy rate.

Considering 15 pregnancies after AIH, there were 46% (n=6) term, spontaneous deliveries, and 33% (n=3) term cesarean sections. Sixty percent of pregnancies ended at term(n=9), while 20% ended with premature delivery (n=3) . There were also 6.7% (n=1) miscarriage rate. Two pregnancies (13,3%) are ongoing. Among patients that achieved a pregnancy by AIH, there was 13.3% (n=3) twin pregnancy rate.

Discussion

In recent update World Health Organization have published new guidelines for evaluation of human sperm [4]. The semen parameters are a surrogate measure of male fecundity, and as such they server as prognostic factors determining the potential for pregnancy and the need for additional methods to increase the chances of achieving pregnancy in situations where the semen is suboptimal. The reasons for recent change in the normal parameters for sperm assessment were: inconsistencies in reference populations and substandard methodologies used by some laboratories [4]. Recent study which assess semen analysis in different laboratories in Poland revealed significant differences between the laboratories in the obtained results of the analysis of sperm parameters, especially the morphology [5]. So the recent guidelines are more “relaxed”, allowing men that have been previously viewed as having poor sperm quality to be now considered as having normal sperm parameters.

This paper describes the results of IUI with husband sperm in our clinic with references according to the latest 2010 WHO criteria described in the 5th edition of the WHO Laboratory Manual for the Examination and Processing of Human Semen [2].

Mateusz Mikołajczyk, et al. *The efficiency of artificial insemination by husband sperm in infertile couples according to the revised WHO 2010 criteria.*

The intrauterine insemination is by far the cheapest, and therefore most accessible method of archiving pregnancy in patients with idiopathic infertility and poor sperm parameters compared to IVF. In a setting where the IVF procedure is paid in full by the patient, many of them opt for this method.

The criteria for sperm grading have changed recently. There were many causes for this less stringent criteria, that describe what is now considered normal sperm parameters. Also some couples despite normal findings in infertility tests, still suffer from so called idiopathic infertility. Those couples despite having a chance for spontaneous conception, might also be candidates for intrauterine insemination.

The success of IUI is very variable according to studies, ranging from 2-60% [6-8]. In our study the cumulative pregnancy rate was 33%, with 11% for a single IUI. This is a very good result considering that most (80%) of our patients had abnormal sperm parameters on initial evaluation. In other Polish population study, the cumulative pregnancy rate was only 7%, with 4% for a single IUI [9]. The results of IUI with poor sperm parameters are invariably lower ranging from 3-10% [9]. Considering the number of IUI procedures per pregnancy admittedly all our patients had sperm parameters much higher than those deemed unacceptable for IUI with motile sperm count $<5 \times 10^6$ and $<5\%$ normal spermatozoa. According to some researchers, using computerized sperm analysis, the predictive factors for successful pregnancy were as follows: normal ($>15\%$) sperm morphology before sperm preparation and progressive motility of more than 25% after the preparation [10]. Most of our patients have met these criteria, and the pregnancy rate per cycle of 11% seems in line with current results obtained from literature.

Also most women in our study were below 30 years of age. There is a wide spread agreement that the IUI success is influenced by the age of a women [11]. This might reflect the declining quality of the oocytes, as not only pregnancy rates are lower, but also there is dramatic increase in the miscarriage rate [12]. However the oldest patient achieving pregnancy was a 40 year old woman. The pregnancy ended in delivery of a healthy newborn at term. This indicates that although the statistics are against older women, for a given patient the chance of achieving pregnancy is still a valid notion.

In our study in some cycles we have used clomiphene citrate for ovulation induction. The use of ovulation inducing drugs is an obvious choice in women suffering from anovulation, but also in women with spontaneous ovulation using the drugs to support ovulation seems to confer some benefits. Despite the fact that in some studies monofollicular ovulation has been associated with poor IUI results, we have not observed this phenomenon [13, 14]. Furthermore it is a standard in our institution to aim for monofollicular ovulation induction with low doses of CC (starting from 50 mg) while going for IUI. It seems that our strategy is as successful as induction of at least 2 dominant follicles, as the pregnancy rates achieved in our paper was higher (11%) than in studies that have used multifollicular ovulation induction (9%) [14].

We did notice a trend for better pregnancy rates in patients using ovulation induction, than in those that did just IUI. A recent Cochrane review has focused on the effects of ovarian stimulation with IUI compared to timed intercourse or just IUI [15]. It has shown that addition of ovulation induction to IUI increases

pregnancy rates. The reasons for that are not fully elucidated. Some believe that CC effect on pregnancy rates depends only on the ability to produce multifollicular development. Also one must not forget that the use of CC is known for its poor influence on the endometrium in some women [16]. Despite these reservations the CC still is the most widely used ovulation enhancing drug, due to its safety profile, ease of administration and monitoring. The more favorable pregnancy rates achieved with CC stimulation in our study convince us to continue with the monofollicular ovulation induction with clomiphene citrate.

Also, in our study some women, despite normal results of diagnostic test and normal sperm parameters, still could not achieve pregnancy spontaneously. After entering our program 15 of them, despite having numerous IUI, achieved pregnancy by natural conception. One could assume that with enough time these women and their partners would achieve pregnancy without the need for entering the IUI program. However there is emerging evidence that endometrial injury (either from the insemination catheter, hysteroscopy or hysterosalpinography) might improve the pregnancy rates in cycle even long time after the procedure was performed [18, 19]. Some couples, do not wish to wait for pregnancy for a prolonged period of time, due to for example pressure from their families, and opt for IUI, despite having normal test results.

The mean duration of infertility in a group with normal sperm parameters was slightly higher (3 years) compared to all patients (2,3 years), as those patients were allowed more time to conceive spontaneously, since there was nothing "wrong" with them, and they were young. So the pregnancies achieved by spontaneous conception might have been a result of more time given to the patient or some, as yet undiscovered, result of endometrial stimulation. There are numerous papers that point to worse pregnancy rates with longer duration of infertility [11, 14]. The duration of infertility in our study was quite short (2.3 years), therefore we cannot draw meaningful conclusions in this matter.

The number of miscarriages (6.2%) was quite low in our study. The number was slightly higher for pregnancies achieved by IUI (6.7%), but still was far better than those by other researchers [19]. It is very difficult to compare the miscarriage rate between various studies, due to differences in couple selection (age, ethnicity, duration of infertility), ovarian stimulation protocols used (different drugs, different dosing) and finally the sperm parameters used in the IUI. The rate for miscarriage in our study was lower compared to miscarriage rate observed in our general population (15%) [20]. It is a well-known fact that CC, due to its anti-estrogenic effect might influence endometrial development and influence miscarriage rates. However in some studies, it has been shown that, after controlling for early pregnancy loss, advanced maternal age, the infertility status, and the increased incidence of multiple pregnancies, there does not seem to be a detrimental effect of CC on miscarriage [21]. The IUI itself has little if any effect on the miscarriage rate [22]. Our mild ovarian stimulation protocol and IUI criteria have ensured low miscarriage rate regardless of the conception type (IUI or spontaneous).

On the other hand we have encountered an unusually high premature delivery rate (12.5%). All of the prematurely delivered infants survived, as the pregnancies ended after 34th week of gestation. In general population the rate of premature deliveries is 6.8% [20]. It is plausible to assume that couples presenting with

Mateusz Mikołajczyk, et al. *The efficiency of artificial insemination by husband sperm in infertile couples according to the revised WHO 2010 criteria.*

infertility, despite normal result of diagnostic test, have some underlying problem either with spermatozoa or with the uterus/endometrium. There is growing evidence that some molecular factors might affect not only the pregnancy rates, but also miscarriage and preterm delivery rates [23, 24]. Currently there is growing awareness of the importance of the endometrium in the implantation process, but still clear guidelines to assess receptive are missing [25].

The rate of twin pregnancies (13%) achieved during the IUI protocols, is well in line with the recently published results by ESHRE [26]. This represents more than 4 fold increase over the normal (3.3%) occurrence rate for twin pregnancies [27]. There are numerous explanations for this phenomenon. Age of a women, use of ovulation induction, use of IUI or other assisted reproductive technology (ART) have been blamed on the rising epidemic of the multiple births [28]. In our study all women with twin pregnancies gave pre term birth after 34th week of gestation: two of them as spontaneous delivery and one had cesarean section.

Conclusions

In conclusion our study seems to support the new criteria for assessment of sperm parameters. Judging the sperm according to the new, lessened criteria, did produce comparable pregnancy rates as with historical cohorts based on old criteria. Therefore the new criteria allow for estimating the spontaneous chances for achieving pregnancy, and might serve as a guideline for helping to choose the right method to help those patients that have suboptimal sperm parameters.

Oświadczenie autorów:

1. Mateusz Mikołajczyk 45% - autor koncepcji i założeń pracy, przygotowanie manuskryptu i piśmiennictwa – autor zgłaszający – odpowiedzialny za manuskrypt.
2. Tomasz Goździewicz 45% – zebranie materiału, analiza statystyczna wyników, przygotowanie manuskryptu.
3. Jana Skrzypczak 10% – ostateczna weryfikacja i akceptacja manuskryptu.

Źródło finansowania: jest to praca retrospektywna, która nie wymagała żadnego wkładu finansowego.

References

1. Mascarenhas M, Flaxman S, Boerma T, [et al.]. National, Regional, and Global Trends in Infertility Prevalence Since 1990: A Systematic Analysis of 277 Health Surveys. *PLoS Med.* 2012, 9, 12: e1001356. doi:10.1371/journal.pmed.1001356.
2. WHO laboratory manual for the Examination and processing of human semen. FIFTH EDITION. WHO. 2010.
3. Ray A, Shah A, Gudi A. Unexplained infertility: an update and review of practice. *Reprod Biomed Online.* 2012, 24, 591-602.
4. Cooper T, Noonan E, von Eckardstein S, [et al.]. World Health Organization reference values for human semen characteristics. *Hum Reprod Update.* 2010, 16, 231-245.
5. Jędrzejczak P, Talarczyk J, Taszarek-Hauke G, [et al.]. External quality assessment of semen analysis in Poland. *Ginekol Pol.* 2012, 83, 835-840.
6. Paulmyer-Lacroix O, Molle L, Noizet A, [et al.]. Intrauterine insemination with the husband's sperm: conclusions of five years experience. *Contracept Fertil Sex.* 1998, 26, 300-306.
7. Andersen A, Gianaroli L, Felberbaum R, [et al.]. Assisted reproductive technology in Europe, 2002. Results generated from European registers by ESHRE. *Hum Reprod.* 2006, 21, 1680-1697.
8. Allen NC, Herbert CM 3rd, Maxson WS et al. Intrauterine insemination: a critical review. *Fertil Steril* 1985;44:569-80.
9. Fritz M, Speroff L. *Clinical Gynecologic Endocrinology and Infertility. Chapter Male Infertility*, 2010,1282.
10. Shihabara H, Obara H, Ayustawati Hirano Y, [et al.]. Prediction of pregnancy by intrauterine insemination using CASA estimates and strict criteria in patients with male factor infertility. *Int J Androl.* 2004, 27, 63-68.
11. Wang B, Hu Y, Sun H, [et al.]. Investigation of correlative factors affecting successful intrauterine insemination. *Zhonghua Nan Ke Xue.* 2004, 10, 526-529.
12. Corsan G, Trias A, Trout S, [et al.]. Ovulation induction combined with intrauterine insemination in women 40 years of age and older: is it worthwhile? *Hum Reprod.* 1996, 11, 1109-1112.
13. Plosker S, Jacobson W, Amato P. Predicting and optimizing success in an intra-uterine insemination programme. *Hum Reprod.* 1994, 9, 2014-2021.
14. Ibérico G, Vioque J, Ariza N, [et al.]. Analysis of factors influencing pregnancy rates in homologous intrauterine insemination. *Fertil Steril.* 2004, 81, 1308-1313.
15. Veltman-Verhulst S, Cohen B, Hughes E, [et al.]. Intra-uterine insemination for unexplained subfertility. *Cochrane Database Syst Rev.* 2012 Sep 12;9:CD001838. doi: 10.1002/14651858.CD001838.pub4. Review.
16. Gorlitsky G, Speroff L. Ovulation and pregnancy rates with clomiphene citrate. *Obstet Gynecol.* 1978, 51, 265-269.
17. Huang S, Wang C, Soong Y, [et al.]. Site-specific endometrial injury improves implantation and pregnancy in patients with repeated implantation failures. *Reprod Biol Endocrinol.* 2011, 9, 140.
18. Karayalgin R, Ozyer S, Ozcan S, [et al.]. Office hysteroscopy improves pregnancy rates following IVF. *Reprod Biomed Online.* 2012, 25, 261-266.
19. Mathieu C, Ecochard R, Bied V, [et al.]. Cumulative conception rate following intrauterine artificial insemination with husband's spermatozoa: influence of husband's age. *Hum Reprod.* 1995, 10, 1090-1097.
20. Niemiec T. Raport - Zdrowie kobiet w wieku prokreacyjnym 15-49 lat. Polska 2006. Program Narodów Zjednoczonych ds. Rozwoju i Ministerstwo Zdrowia, Warszawa 2007.
21. Shoham Z, Zosmer A, Insler V. Early miscarriage and fetal malformations after induction of ovulation (by clomiphene citrate and/or human menopausal gonadotropins), in vitro fertilization, and gamete intrafallopian transfer. *Fertil Steril.* 1991, 55, 1-11.
22. Ferraretti A, Goossens V, de Mouzon J, [et al.]. European IVF-monitoring (EIM); Consortium for European Society of Human Reproduction and Embryology (ESHRE). *Hum Reprod.* 2012, 27, 2571-2584.
23. Pinborg A, Wennerholm U, Romundstad L, [et al.]. Why do singletons conceived after assisted reproduction technology have adverse perinatal outcome? Systematic review and meta-analysis. *Hum Reprod Update.* 2012, 14.
24. Filicori M, Cognigni G, Gamberini E, [et al.]. Impact of medically assisted fertility on preterm birth. *BJOG.* 2005, 112, Suppl 1, 113-117.
25. Brosens J, Hodgetts A, Feroze-Zaidi F, [et al.]. Proteomic analysis of endometrium from fertile and infertile patients suggests a role for apolipoprotein A-I in embryo implantation failure and endometriosis. *Mol Hum Reprod.* 2010, 16, 273-285.
26. ESHRE Capri Workshop Group. Intrauterine insemination. *Hum Reprod Update.* 2009, 15, 265-277.
27. Bush M, Pernoll M. Multiple Pregnancy. *McGraw Hill Professional.* 2006.
28. Dudenhausen J, Maier R. Perinatal Problems in Multiple. *Dtsch Arztebl Int.* 2010, 107, 663-668.