Outcome of Subsequent IVF Cycles after Antibiotic Therapy Following Previously Failed IVF Cycles. Study II

ATTILA TOTH[1,2], ADDISON B. TOTH

[1]MacLeod Laboratory and [2]New York Presbyterian Medical Center, New York, USA

Objective: To determine whether broad-spectrum antibiotic therapy administered after a failed *in vitro* fertilization (IVF) cycle will improve the chance of achieving a successful pregnancy in the subsequent IVF cycle and to determine whether further antibiotic therapy administered immediately after conception, during the course of pregnancy, and at the time of delivery will reduce the incidence of maternal and fetal complications. *Design:* A retrospective analysis was performed on the clinical data of 63 couples who had previously failed one or more IVF cycles and were subsequently treated with broad-spectrum antibiotics. All women were treated with intravenous Clindamycin and daily intrauterine lavages using a broad-spectrum antibiotic combination. All men received intravenous Clindamycin and simultaneously underwent five direct transrectal injections of an antibiotic cocktail into the prostate gland if clinical or sonographic evidence of chronic prostatitis was documented. For both men and women, these regimens were followed by a month-long oral course of Zithromax and Macrobid. Following either a spontaneous pregnancy or a repeat IVF conception, a 10-day antibiotic course was administered in the form of oral Cleocine or intravenous Clindamycin. Some women received intermittent oral antibiotic courses throughout the pregnancy and prophylactic antibiotics during delivery. *Results:* When compared with our previous study (Study I, [24]), the number of spontaneous pregnancies was significantly higher and the total number of births was also significantly higher. Following the antibiotic therapy, there was a significantly improved chance for the couples to achieve a successful IVF pregnancy when compared to historical controls in conventional repeat IVF cycles. For singleton pregnancies, there were no perinatal maternal or fetal complications. *Conclusion:* These results support our previous findings, showing that antibiotic therapy can reduce IVF failures in subsequent cycles. The reduced number of maternal and fetal complications after antibiotic therapy in this retrospective study is impressive and warrants a prospective, randomized trial for confirmation.

Keywords: cumulative pregnancies, IVF, antibiotics and IVF, perinatal complications and antibiotics, IVF failure and infections

Abbreviations

A7 Differential agar = a solid medium used for detection and identification of ureaplasma urealyticum and mycoplasma; API system = (active pharmaceutical ingredient), a multitube micro method for identification of bacteria and yeast; BD test = Breslow–Day test, test of homogeneous association; CI = confidence interval; GA = gestational age; IVF = *in vitro* fertilization; IUGR = intrauterine growth restriction; MH test = Mantel–Haenszel statistical test. The purpose of the MH is to estimate the average conditional association between the explanatory and the response variable; NICU = newborn intensive care unit; *P*-value = probability; PCR = polymerase chain reaction; Rapid-NH = specialized media for gonorrhea and hemophilus identification; PROM = premature rupture of membranes; RCRC Institutional Review Board = Reviews the merit of a scientific work before submission for publication; SD = standard deviation; TV pouch = liquid medium for *Trichomonas vaginalis* culture

Corresponding address: Attila Toth, Director, MacLeod Laboratory, Associate Clinical Professor, New York Presbyterian Medical Center, 65 East 79th Street, New York, NY 10075, USA. Phone: 212 717 4444, Fax: 212717 1868. E-mailes: tiltoth@aol.com, http://www.fertilitysolution.com

Introduction

In developed countries, assisted reproductive technologies (ART), mostly *in vitro* fertilization (IVF) deliveries, account for up to 4% of total deliveries [1]. Since the birth of the first IVF baby in 1978, well over a million babies worldwide have been born after IVF [2]. While improvements in IVF technology have offered numerous infertile couples the chance to achieve a successful pregnancy, the per cycle success rate in most clinics hovers around 25% [3, 4] and the chance for a successful outcome diminishes with subsequent trials. The cumulative live birth rate after seven IVF cycles is around 60% [5–8]. Some of the failures are attributed to the male factor [9], poor oocyte quality [10], reduced ovarian reserve [11], uterine problems [12], and chromosomal abnormalities of the embryos [13]. In general, advanced maternal age is associated with a poorer outcome [14]. The role of infections is also appreciated, and most IVF cycles are complemented with a limited oral antibiotic regimen [15–18]. In a high percentage of cases, however, the cause of failure remains unknown. In addition, there are only speculative explanations why IVF pregnancies are more often complicated by premature birth, intrauterine growth retardation (IUGR), and chromosomal abnormalities than naturally conceived pregnancies [19–22]. The affected children have long-term, expensive-to-manage medical problems that force us to refocus our attention on the possible etiologies of these conditions.

Our laboratory has three decades of favorable experience with the use of antibiotic therapy in reversing infertility. In addition to reversing infertility, we have witnessed improved pregnancy outcomes and a reduction in perinatal maternal and fetal complications. Initially, we administered antibiotics orally [22, 23]. Later, the oral therapy was replaced with intravenous antibiotics in combination with uterine lavages using antibiotic cocktails. This regimen also proved to be beneficial in improving the chance of achieving subsequent pregnancies for women whose previous IVF cycles had been unsuccessful. Pregnancy-related maternal and fetal complications associated with IVF cycles were also significantly reduced [24]. Recognizing the impact that infections within the male genital canal can have on a couple's fertility, we recommend IV antibiotics and direct injections of an antibiotic cocktail into the prostate of all men with clinical and sonographic evidence of chronic prostatitis. It is exceedingly rare to find negative semen cultures in men suffering from chronic prostatitis. Bacteria harbored in the inflamed prostate are transferred into the woman and can cause infertility and structural damage. Similarly, an abnormal bacterial flora in the vagina will find its way into the male prostate and will lead to chronic prostate infection. There are numerous publications supporting the beneficial effects of antibiotics directly injected into the prostate. It is safely used and is no longer considered experimental [25–30].

We report our experience here with 63 couples that were treated at our clinic between January 1, 2006 and January 1, 2009.

Materials and Methods

Study Design

A retrospective chart analysis and telephone follow-up was carried out on 63 couples that were treated at the MacLeod Laboratory between January 1, 2006 and January 1, 2009.

RCRC Institutional Review Board approved the study. Informed consent was obtained from the patients for data collection, analysis, and publication conforming to local and national regulations. The authors had full access to the data, directed the data analysis, and were responsible for decisions regarding publication. The principal investigator (Dr. Toth) assumes full responsibility for the integrity and interpretation of the data.

Patients

A total of 63 consecutive couples referred to us for antibiotic therapy with a history of primary or multiple previously failed IVF cycles were eligible for the study. Cervical and endometrial cultures on the women and seminal fluid and urethral cultures on the men were performed before the antibiotic therapy was initiated. *Chlamydia trachomatis* was tested using the Pathfinder Direct Antigen Detection System from Bio-Rad Laboratories, A7 differential agar was used to identify Mycoplasma, and API systems were used for aerobic bacterial identification. A Ramel Rapid Ana II system was used to identify anaerobic bacteria, and the API 20c AUX system was used to identify yeast. The Rapid NH System identified Neisseria and Hemophilus. *Trichomonas vaginalis* was identified after overnight growth in a selective broth (in a Pouch TV test kit). The result of the culture studies did not influence the recommendation of antibiotic therapy. All women were treated with a combination of 10 days of intravenous Clindamycin in a full therapeutic dose, typically 900 mg every 8 h for an individual of 150 lbs body weight, and 10 intrauterine lavages performed on consecutive days. The lavages applied a mixture of 6 g ampicillin, 160 mg gentamicin, 4 mg fluconazole (Diflucan), and 50 mg Medrol in a 1-h daily infusion using an ambulatory pump and a Cook 5.3FR intrauterine catheter. At the end of each lavage, the uterine cavity and cervical canal were filled with a 20% metronidazole-containing gel prepared by a local pharmacy. Similarly, intravenous Clindamycin was given to all men for a 10-day duration. For those men exhibiting clinical, laboratory, or sonographic evidence of chronic prostatitis, on alternate days a cocktail of broad-spectrum antibiotics was injected into the prostate gland using sonographic guidance through a transrectal approach. The antibiotic cocktail (a total volume of 10 ml) contained 150 mg Clindamycin, 80 mg gentamicin, 10 mg metronidazole (Flagyl), 50 mg Levaquin, 50 mg Zithromax, 4 mg fluconazole (Diflucan), and 50 mg methylprednisolone (Medrol). Out of the 63 couples, 42 men were treated with direct injections. For both men and women, this initial 10-day regimen was followed by a one-month long oral Zithromax, 500 mg once daily, and Macrobid (macrodantin), 100 mg twice-daily, combination antibiotic regimen. Except for two cases of mild diarrhea, which responded promptly to oral metronidazole, and one case of moderate skin rash (treated with oral prednisone, 10 mg three times daily for 5 days), no other complications were encountered with any of the antibiotic treatment modalities. The uterine lavages were performed without any complications. Transient hemospermia and hematuria, lasting up to 4 weeks, were the only complications associated with the transrectal injections. If a spontaneous or IVF pregnancy occurred following the antibiotic therapy, cervical smears were tested for Chlamydia elementary bodies 2 and 4 months following conception. If elementary bodies were detected, alternating 10-day oral Cleocine courses (600 mg three times daily and Zithromax 500 mg once daily) were given to the patients in 2-month intervals throughout the pregnancy. These patients received prophylactic intravenous Clindamycin during delivery, one dose of 900 mg prior to a scheduled ce-

sarean section, or, for spontaneous delivery, the same dose repeated every 8 h throughout labor and delivery.

Study Procedure and End Points

Reports on reproductive events following the completion of the antibiotic therapy were gathered via a direct telephone interview after a successful delivery or, if a successful pregnancy did not occur, up to a maximum of 18 months.

Statistical Methods

The primary statistical analyses were comparisons of the delivery rates for the antibiotic-treated patients with those of two different historical control samples of "conventional" IVF patients [7, 8]. This was accomplished using the Mantel–Haenszel (MH) test, stratified according to the number of previously failed cycles. More specifically, each of the published manuscripts contained tables showing the number of patients entering a given IVF cycle and the number who delivered immediately after that cycle. The number of previously failed cycles was the current cycle minus 1. Based on this information, multiple 2×2 contingency tables comparing the delivery frequency of the antibiotic-treated sample with the particular historical sample were formed, each table corresponding to (i.e. stratified for) the number of failed cycles. Only patients with five or fewer failed cycles were included in the analysis, since the data were too sparse for six or more cycles. In order for a delivery outcome to be counted as a "success", the delivery had to occur in the immediately subsequent IVF cycle (as reported in the literature).

The standard MH test was used, first checking for homogeneity of the odds ratios using the Breslow–Day (BD) test (SAS Version 9.1, SAS Institute, Cary, NC). In all reported analyses, the BD test was nonsignificant (P-values of 0.61 and 0.56), thus allowing for "combining" the stratified 2×2 tables according to the MH method. Due to the sparseness of many of the tables, exact 95% confidence intervals were also computed, which were nearly identical to the asymptotic results; only the asymptotic results are presented. Results are reported in terms of relative risk (RR) and its associated 95% confidence interval (CI). In this report, RR represents the "risk" of a successful delivery for the antibiotic-treated group relative to the particular conventional IVF control. Accordingly, RR >1 is favorable to the antibiotic-treated group. A result was considered statistically significant if $P < 0.05$.

Results

Comparisons of Ages

There were 50 patients who had had five or fewer prior IVF failures and then received antibiotic therapy; of these, 23 pursued another cycle of IVF after antibiotic treatment. The 23 patients' mean age was 38.7 (±4.5 SD) years. The mean ages for the Malizia and Elizur studies were 35.8 (±4.7) and 32.7 (±5.9) years, respectively.

Comparisons of Delivery Rates

The data used in the MH test calculations are shown in *Table 1*.

In both comparisons to the historical controls, the antibiotic-treated group had significantly higher delivery rates than the controls, as follows: When compared to the patients in Malizia's report, those receiving antibiotics were 3.9 times more likely to deliver on the current cycle than the controls ($P < 0.0001$, RR = 3.9, 95% CI: 3.1–5.0). When compared to the controls in the Elizur publication, the delivery rate was 6.6 times greater (P < 0.0001, RR = 6.6, 95% CI: 5.0–8.6)

Similar statistical calculations were performed after combining the data from our first study (Study I, 52 treated couples, mean age 38.1 (±4.1 SD) years, [24]) with the recent study (Study II, 63 treated couples) on a total of 105 cases. All these couples experienced one or multiple IVF failures before undergoing antibiotic therapy. Again, only cases with fewer than five previous failed IVF cycles were included and, in order for a delivery outcome to be counted as a "success", the delivery had to occur in the immediately subsequent IVF cycle (as reported in the literature).

In Studies I and II combined, there were 94 couples who had five or fewer prior IVF failures and then received antibiotic therapy; of these 94 couples, 45 pursued another cycle of IVF after antibiotic treatment.

Comparisons of Singleton Delivery Rates to historical controls: Combined data from Study I and Study II.

The data used in the MH test calculations are shown in *Table 2*.

In both comparisons to the historical controls, the antibiotic-treated group had significantly higher delivery rates than the historical controls, as follows: When compared with the

Table 1 Comparing two historical controls for chance of delivery (relative risk) after antibiotic therapy: Study II

No. of prior failures	Antibiotic (Toth)		Malizia		Antibiotic (Toth)		Elizur	
	Delivery		Delivery		Delivery		Delivery	
	Yes	No	Yes	No	Yes	No	Yes	No
1	4	0	784	3053	4	0	148	1015
2	4	2	475	1753	4	2	92	657
3	3	2	221	949	3	2	50	435
4	5	1	99	474	5	1	32	300
5	1	1	36	240	1	1	29	214
Total	17	6	1615	6469	17	6	351	2621
Crude delivery rate (%)	73.9		20.0		73.9		11.8	
MH relative risk	RR = 3.9; 95% CI: 3.1–5.0; P < 0.0001				RR = 6.6; 95% CI: 5.0–8.6; P < 0.0001			

Table 2 Comparing two historical groups for chance of delivery (relative risk) in the combined group after antibiotic therapy. In Study I (22) and in Study II (23) repeated IVF, a total of 45 women

No. of prior failures	Antibiotic (Toth)		Malizia		Antibiotic (Toth)		Elizur	
	Delivery		Delivery		Delivery		Delivery	
	Yes	No	Yes	No	Yes	No	Yes	No
1	9	1	784	3053	9	1	148	1015
2	5	5	475	1753	5	5	92	657
3	7	4	221	949	7	4	50	435
4	9	2	99	474	9	2	32	300
5	1	2	36	240	1	2	29	214
Total	31	14	1615	6469	31	14	351	2621
Crude delivery rate (%)	68.9		20.0		68.9		11.8	
MH relative risk	RR = 3.6; 95% CI: 3.0–4.4; P < 0.0001				RR = 6.1; 95% CI: 4.9–7.7; P < 0.0001			

Table 3 Summary table of number of couples and pregnancies in Study I and Study II

Pregnancy type	Study		
Frequency Col Pct	Study I	Study II	Total
No. of deliveries	29	28	57
	44.23	55.55	
Twins	4	7	11
	7.69	11.11	
Spontaneous singleton	3	12	15
	5.77	19.05	
Singleton first IVF post antibiotic therapy	14	16	30
	26.92	25.40	
Singleton second IVF post antibiotic therapy	2	0	2
	3.85	0.00	
Total number of couples	52	63	115

Table 4 Comparison of birth weight (WT) and gestational age (GA) of IVF deliveries: Study I and Study II

Study	N Obs	Variable	Label	N	Mean	Median	SD	Lower quartile	Upper quartile
Study I	14	GA	GA	14	272.6	276.0	15.1	259.0	287.0
		WT	WT	14	3477.2	3674.0	461.3	3010.0	3883.0
Study I	16	GA	GA	16	276.0	275.0	6.9	271.0	283.0
		WT	WT	16	3526.4	3414.5	607.2	3101.0	3784.5

patients in Malizia's report, those receiving antibiotics were 3.6 times more likely to deliver on the current cycle than the controls ($P < 0.0001$, RR=3.6, 95% CI: 3.0–4.4). When compared with the controls in the Elizur publication, the delivery rate was 6.1 times greater ($P < 0.0001$, RR = 6.1, 95% CI: 4.9–7.7) *(Table 3)*.

Comparisons of Singleton Delivery Rates: Study I and Study II.

With the more intensive antibiotic therapy, there was a significantly higher chance for delivery (35 vs. 23). The rate of spontaneous pregnancy in the second study group was significantly higher (12 in Study II vs. 3 in Study I). There was no significant difference in birth weight between the two studies *(Table 4)*. There was a trend for deliveries to occur closer to the ideal 280 days after the administration of broader spectrum antibiotics to the male partner in Study II. This difference, however, did not reach statistical significance *(Fig. 1)*.

Discussion

Published clinical trials showing either the beneficial effect of antibiotics or no effect at all on improving IVF pregnancy rates all used limited courses of orally administered antibiotics [31, 32]. There are no studies evaluating the effects of broad-spectrum antibiotics given to couples who failed IVF cycle or to evaluate the benefit of antibiotics in reducing maternal and fetal complications known to be increased after IVF. Numerous studies promote the use of different antibiotics for selected pathogens, or for delaying delivery after the onset of premature labor, or to prevent infectious complications after premature rupture of membranes or following cesarean section [33–36]. Previous works from our laboratory showed that preconceptional antibiotic therapy, initially administered orally [22, 23] and later intravenously with the addition of uterine washes with broad-spectrum antibiotics, greatly improved a woman's fertility, reduced the chance of a repeat miscarriage, and facilitated the delivery of full-term, healthy newborns without maternal or fetal complications. In addition, broad-spectrum antibiotics given intravenously combined with intrauterine lavages greatly improved a woman's chance to achieve a subsequent spontaneous or IVF pregnancy after one or multiple previously failed IVF cycles (Study I, [24]).

The current study reports our latest findings on 63 couples that experienced one or multiple IVF failures before consulting with us for antibiotic therapy and represents the continuation of a management philosophy of infertility based on an infectious etiology. The major difference between Study I and the current study (Study II) is the appreciation of the
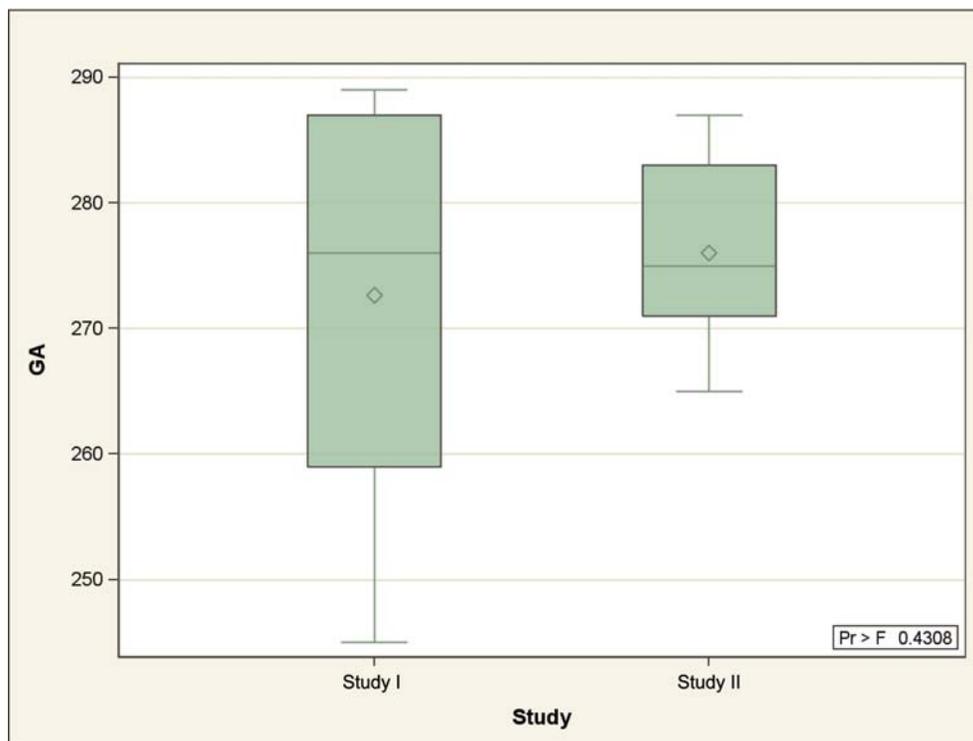
Fig. 1 Comparison of gestational age (GA in days) at delivery: Study I and Study II

male factor in infertility and pregnancy-related complications: more precisely, the role of bacteria harbored in the male genital tract and their effect on sperm function, female fertility, and pregnancy complications. The prime reservoir for these bacteria is the prostate gland. It has been shown that direct antibiotic injection into the prostate is by far more efficient in reducing bacterial colony count in the semen than orally administered antibiotics. Our experience with chronic prostatitis shows that, in over half of these patients, a urethral swab is positive for Chlamydia elementary bodies detectable by fluorescent antibody staining. The high isolation rate of Chlamydia in our patient population is troublesome and warrants explanation. We have encountered several patients whose IVF was initiated without prior Chlamydia screening. In cases where previous screening was performed and therapy was given for Chlamydia infection, we assume the presence of a resistant strain or a mutated strain that is no longer detectable by polymerase chain reaction (PCR), rather than a reinfection. We are aware of the emergence of multidrug resistant Chlamydia strains and encountered a number of cases where long, broad-spectrum antibiotic therapy courses failed to eradicate Chlamydia [37, 38]. A significant number of patients with chronic prostatitis will reveal a variety of heavily growing anaerobic bacteria. Therefore, all men with clinical, laboratory, or sono-

graphic evidence of chronic prostatitis were given five direct transrectal antibiotic injections using the described cocktail. Neither the pretreatment Chlamydia-positive culture status nor the total number of pretreatment bacterial isolates was a predictor of a successful pregnancy. The small sample size, however, prevents a definite conclusion. Clearly, more detailed microbiological studies are indicated. In general, IVF pregnancies have an increased risk of developing pregnancy-associated complications, such as bleeding, pre-eclampsia, placenta previa, premature rupture of membranes (PROM), and preterm delivery. Interventions, including cesarean sections and induction of labor, are more frequent. The newborns conceived through an IVF cycle have a higher chance of being extremely low or low birth weight and suffer from IUGR and congenital malformations [39–42]. In our Study II, none of the patients delivered prematurely and none of the babies was of small birth weight and there were no intra-uterine-growth-retarded newborns. Our series does not include complicated pregnancies and the deliveries of sickly children. Newborn intensive care unit (NICU) admissions among singleton deliveries were for observation only: 3 days for women who conceived spontaneously after antibiotic therapy and 7 days for women who delivered after an IVF procedure. There were significant differences between Study I and Study II. In Study II, the total number of deliveries was significantly higher, and significantly more couples achieved spontaneous pregnancies. The birth weight in the two studies did not differ significantly. In the second study, there was a trend for the deliveries to come close to the ideal 280 days. This difference, however, did not reach statistical significance. Despite the failure of microbiological studies to show a difference between those patients who failed or succeeded in subsequent IVF cycles, we attribute this favorable outcome to the antimicrobial effect of the antibiotics and postulate that, where IVF pregnancies are associated with premature delivery, IUGR, extreme prematurity, maternal and fetal infectious complications, and the need for NICU admission, an intrauterine infection is at play.

We recognize the limitations of a retrospective study. The small sample size, potential bias due to patient selection in a unique, private practice setting, the previously failed IVF cycle as prerequisite to be enrolled into the study, all could have influenced the outcome. Still, the uniform beneficial effect of antibiotics in improving the chance for a pregnancy in the subsequent IVF cycle as well as the reduced number of maternal and fetal complications in the post antibiotic pregnancies prompts us to put forward our findings and urge the medical community to take part in a prospective, randomized trial where these observation can be put to a final test.

Acknowledgments

References

[1] *Klemetti, R., Gissler, M., Hemminiki, E.:* Comparison of perinatal health of children born from IVF in Finland in the early and late 1990s. Hum. Reprod., 2002, *17,* 2192–2198.

[2] *Bondurlle, M., Wennerholm U. B., Loft, A. et al.:* A multi-center cohort study of the physical health of 5-year-old children conceived after intracytoplasmic sperm injection, in vitro fertilization and natural conception. Hum. Reprod., 2005, *20,* 413–419.

[3] *Schroder, A. K., Katalinic, A., Diedrich, K., Ludwig, M.:* Cumulative pregnancy rates and drop-out rates in a German IV program: 4102 cycles in 2130 patients. Reprod. Biomed. Online 2004, *8,* 600–606.

[4] *Sharma, V., Allgar, V., Rajkhowa, M.:* Factors influencing the cumulative conception rate and discontinuation of in vitro fertilization treatment for infertility. Fertil. Steril., 2002, *78,* 40–46.

[5] *Witsenburg, C., Dieben, S., Van der Westerlaken, L., Verburg, H., Naaktgeboren, N.:* Cumulative live birth in cohorts of patients treated with in vitro fertilization or intracytoplasmic sperm injection. Fertil. Steril., 2005, *84,* 99–107.

[6] *Bartoov, B., Berkovitz, A., Eltes, F., Kogosovsky, A., Yagoda, A., Lederman, H., Artzi, S., Gross, M., Barak, Y.:* Pregnancy rates are higher with intracytoplasmic morphologically selected sperms then with conventional intracytoplasmic injection. Fertil. Steril., 2003, *80,* 1413–1419.

[7] *Malizia, B. A., Hacker, M. R., Penzias, A. S.:* Cumulative live-birth rates after in vitro fertilization. N. Engl. J. Med., 2009, *360,* 236–243.

[8] *Elizur, S. E., Lerner-Geva, L., Levron, J., Shulman, A., Bider, D., Dor, J.:* Cumulative live birth rate following in vitro fertilization: study of 5,310 cycles. Gynecol. Endocrinol., 2006, *22*(1), 25–30.

[9] *Sharma, V., Allgar, V., Rajkhowa, M.:* Factors influencing the cumulative conception rate and discontinuation of in vitro fertilization treatment for infertility. Fertil. Steril., 2002, *78,* 40–46.

[10] *Navot, D., Bergh, P. A., Williams, M. A. et al.:* Poor oocytes quality rather than implantation failure as a cause of age-related decline in female fertility. Lancet, 1991, *337,* 1375–1377.

[11] *Wallach, E. E.:* Pitfalls in evaluating ovarian reserve. Fertil. Steril., 1995, *63,* 12–14.

[12] *Abdalla, H. I., Baber, R., Kirkland, A., Leonard, T., Power, M., Studd, J. W.:* A report on 100 cycles of oocytes donation: factors affecting the outcome. Hum. Reprod., 1990, *5,* 1018–22.

[13] *Munn, S., Alikani, M., Tomlin, G., Grifo, J., Cohen, J.:* Embryo morphology, developmental rates, and maternal age correlated with chromosome abnormalities. Fertil. Steril., 1995, *64,* 382–391.

[14] *Lass, A., Croucher, C., Duffy, S., Dawson, K., Margara, R., Winston, R. L.:* One thousand initiated cycles of in vitro fertilization in women >40 years of age. Fertil. Steril., 1998, *70,* 1030–1034.

[15] *Romero, R., Espinoza, J., Mazor, M.:* Can endometrial infection/inflammation explain implantation failure, spontaneous abortion, and preterm birth after in vitro fertilization? Fertil. Steril., 2004, *82,* 799–804.

[16] *Witkin, S. S., Kligman, I., Grifo, J. A., Rosenwaks, Z.:* Chlamydia trachomatis detected by polymerase chain reaction in cervices of culture-negative women correlates with adverse in vitro fertilization outcome. J. Infect. Dis., 1995, *171,* 1657–1659.

[17] *Hurst, B. S., Tucker, K. F., Awoniyi, C. A., Schlaff, W. D.:* Hydrosalpinx treated with extended doxycycline does not compromise the success of in vitro fertilization. Fertil. Steril., 2001, *75,* 1017–1019.

[18] *Peikrishvili, R., Evrard, B., Pouly, J. L., Janny, L.:* Prophylactic antibiotic therapy (amoxicillin + clavulenic acid) before embryo transfer for IVF is useless. Results of a randomized study. J. Gynecol. Obstet. Biol. Reprod. (Paris), 2004, *33*(8), 713–719.

[19] *McGovern, P. G., Llorens, A. J., Skurnick, J. H., Weiss, G., Goldsmith, L. T.:* Increased risk of preterm birth in singleton pregnancies resulting from in vitro fertilization-embryo transfer or gamete intrafallopian transfer: a meta-analysis. Fertil. Steril., 2004, *82,* 1514–1520.

[20] *Templeton, A., Morris, J. K., Parslow, W.:* Factors that affect outcome of in-vitro fertilization treatment. Lancet,1 996, *348,* 1402–1406.

[21] *Jackson, R. A., Gibson, K. A., Wu, Y. W., Crougham, M. S.:* Perinatal outcome in singletons following in vitro fertilization: a meta-analysis. Obstet. Gynecol., 2004, *103*(3), 551–563.

[22] *Toth, A., Lesser, M. L., Brooks, C., Labriola, D.:* Subsequent pregnancies among 161 couples treated for T-mycoplasma genital-tract infection. N. Engl. J. Med., 1983, *308,* 505–507.

[23] *Toth, A., Lesser, M. L., Brooks, C.:* Outcome of subsequent pregnancies following antibiotic therapy after primary or multiple miscarriages. Surg. Gyn. Obst., 1986, *163,* 243–250.

[24] *Toth, A., Lesser, M. L.:* Outcome of subsequent IVF cycles following antibiotic therapy after primary or multiple previously failed IVF cycles. Int. J. Gynecol. Obstet., 2007, *7*, 1. http://www.ispub.com/ostia/index. php?xmlFilePath=journals/ijgo/vol7n1/outcome.xml

[25] *Hu, W. L., Zhong, S. Z., He, H. X.:* Treatment of chronic bacterial prostatitis with amikacin through anal submucosal injection. Asian J. Androl., 2002, *4*(3), 163–167.

[26] *Mayersak, J. S.:* Transrectal ultrasonography directed intraprostatic injection of gentamicin-xylocaine in the management of the benign painful prostate syndrome. A report of a 5-year clinical study of 75 patients. Int. Surg., 1998, *83*(4), 347–349.

[27] *Yavaşçaoğlu, I., Camlikiyi, H., Oktay, B., Simşek, Ozyurt, M.:* Percutaneous suprapubic transvesical route: a new and comfortable method of intraprostatic injection. Urol. Int., 1998, *60*(4), 229–234.

[28] *Yamamoto, M., Hibi, H., Satoshi, K., Miyake, K.:* Chronic bacterial prostatitis treated with intraprostatic injection of antibiotics. Scand. J. Urol. Nephrol., 1996, *30*(3), 199–202.

[29] *Guercini, F.:* Chronic prostatitis (CP)/chronic pelvic pain syndrome (CPPS): what do we know? Arch. Ital. Urol. Androl., 2007, *79*(2), 76–83. Review.

[30] *Shoskes, D. A.:* Chronische Prostatitis/chronische. Beckenschmerzsyndrom. Der Urologe, 2009, *48*, 1339–1345.

[31] *Liversedge, N. H., Jenkins, J. M., Keay, S. D. et al.:* Antibiotic treatment based on seminal cultures from asymptomatic male partners in in-vitro fertilization is unnecessary and may be detrimental. Hum. Reprod., 1996, *11*(6), 1227–1231.

[32] *Steyaert, S. R., Leroux-Roels, G. G., Dhont, M.:* Infections in IVF: review and guidelines. Hum. Reprod. Update, 2000, *6*, 432–441.

[33] *Cousens, S., Blencowe, H., Gravett, M., Lawn, J. E.:* Antibiotics for pre-term pre-labour rupture of membranes: prevention of neonatal deaths due to complications of pre-term birth and infection. Int. J. Epidemiol., 2010, *39*(Suppl 1), i134–i143.

[34] *Kalwij, S., Macintosh, M., Baraitser, P.:* Screening and treatment of Chlamydia trachomatis infections. BMJ., 2010, *c1915*, 340, doi: 10.1136/bmj.c1915.

[35] *Bahadi, A., El Kabbaj, D., Elfazazi, H., Abbi, R., Hafidi, M. R., Hassani, M. M., Moussaoui, R., Elouennass, M., Dehayni, M.:* Oualim Z. Urinary tract infection in pregnancy. Saudi. J. Kidney Dis. Transpl., 2010, *21*(2), 342–344.

[36] *Ahmed, Y., Mwaba, P., Grange, J., Green, C., Zumla, A.:* Community-based interventions to reduce maternal mortality. Lancet, 2010, *375*(9713), 457–8; author reply 458–9.

[37] *Misyurina, O. Y., Chipitsyna, E. V., Finashutina, Y. P. et al.:* Mutations in a 23S rRNA gene of Chlamydia trachomatis associated with resistance to macrolides. Antimicrob. Agents Chemother., 2004, *48*, 1347–1349.

[38] *Kutlin, A., Kohlhoff, S., Roblin, P., Hammerschlag, M. R., Riska, P.:* Emergence of resistance to rifampin and rifalazil in Chlamydophila pneumoniae and Chlamydia trachomatis. Antimicrob. Agents Chemother., 2005, *49*, 903–907.

[39] *Kallen, B., Finnstrom, O., Nygren, K. G., Otterblad Olausson, P., Wennerholm, U. B.:* In vitro fertilization in Sweden: obstetric characteristics, maternal morbidity and mortality. BJOG, 2005, *112*, 1529–1535.

[40] *Ombelet, W., Cadron, I., Gerris, J. et al.:* Obstetric and perinatal outcome of 1655 ICSI and 3974 IVF singleton and 1102 ICSI and 2901 IVF twin birth: a comparative analysis. Reprod. Biomed. Online, 2005, *11*, 76–85.

[41] *Maman, E., Lunenfeld, E., Levy, A., Vardi, H., Potashnil, G.:* Obstetric outcome of singleton pregnancies conceived by in vitro fertilization and ovulation induction compared with those conceived spontaneously. Fertil. Steril., 1998, *70*, 240–245.

[42] *Sutcliffe, A. G., Ludwig, M.:* Outcome of assisted reproduction. Lancet, 2007, *370*, 351–359, Review.