

# **Outcome Of Subsequent IVF Cycles After Antibiotic Therapy Following Previously Failed IVF Cycles. Study II.**

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# Abstract

**Objective:** To determine if broad-spectrum antibiotic therapy administered after a failed 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 females were treated with intravenous Clindamycin and daily intrauterine lavages using a broad-spectrum antibiotic combination. All males received intravenous Clindamycin and simultaneously underwent 5 direct trans rectal injections of an antibiotic cocktail into the prostate gland if clinical or sonographic evidence of chronic prostatitis was documented. For both males and females these regimens were followed by a month-

long oral course of Zithromax and Macrobid. Following either a spontaneous pregnancy or repeat IVF conception, a ten-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 to our previous study (Study I, Reference 24) the number of spontaneous pregnancies was significantly higher and the total number of birth 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 findings support our previous hypothesis, which suggested that a large number of IVF failures are due to an intrauterine infection that can affect the course of the pregnancy, the mode of delivery and lead to maternal and fetal complications. We believe the improved results are related to the aggressive therapy of the male partners emphasizing the importance of the male factor.

# Introduction

In developed countries Assisted Reproductive Technologies (ART), mostly 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% (5,6,7,8). Some of the failures are attributed to 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 (15,16,17,18). 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 and chromosomal abnormalities than naturally conceived pregnancies. (19,20,21,22).

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 males 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 female 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 01/01/2006 and 01/01/2009.

# **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 author had full access to the data,

directed the data analysis, and was 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 females and seminal fluid and urethral cultures on the males were performed before the antibiotic therapy was initiated. Chlamydia trachomatis was tested for 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 bacteria identification. 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 Haemophilus. Trichomonas vaginalis was identified after overnight growth in a selective broth. (In Pouch TV test Kit). The result of the culture studies did not influence the recommendation of antibiotic

therapy. All females were treated with a combination of ten days of intravenous Clindamycin in a full therapeutic dose, typically, 900 mg every 8 hours for an individual of 150 lbs body weight, and ten intrauterine lavages performed on consecutive days. The lavages applied a mixture of 6 grams Ampicillin, 160 mgs gentamicin, 4 mgs fluconazole (Diflucan), 50 mgs Medrol in a one-hour daily infusion using an ambulatory pump and a Cook 5.3FR intrauterine catheter. At the end of each lavages 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 males for a ten-day duration. For those males exhibiting clinical, laboratory or sonographic evidence of chronic prostatitis, on alternating days, a cocktail of broad-spectrum antibiotics was injected into the prostate gland using sonographic guidance through a trans rectal approach. The antibiotic cocktail in a total volume of 10 cc. contained 150 mgs Clindamycin, 80 mgs gentamicin, 10 mgs metronidazole (Flagyl), 50 mgs Levaquin, 50 mgs Zithromax, 4 mgs fluconazole (Diflucan), and 50 mgs methylprednisolone (Medrol). Out of the 63 couples 42 males were treated with direct injections. For both males and females this initial ten-day regimen was



followed by a one month long oral Zithromax, 500 mgs once daily and Macrobid (macrodantin) 100 mgs 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 mgs three-times daily for five 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 four 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 two and four month following conception. If elementary bodies were detected, alternating ten-day oral Cleocine courses, 600 mgs three times daily and Zithromax 500 mgs, once daily were given to the patients in two-month intervals throughout the pregnancy. These patients received prophylactic intravenous Clindamycin during delivery, one dose of 900 mgs prior to a scheduled cesarean section or for spontaneous delivery, same dose was repeated every eight hour 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 statistical analysis was performed by:

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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 (Malizia et al., 2009; Elizur et al., 2006). 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 one. Based on this information, multiple 2x2 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 non-significant (P-values of 0.61 and 0.56), thus allowing for "combining" the stratified 2x2 tables according to the MH method. Due to the sparseness of many of the tables, exact 95% confidence intervals were also computed and 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 toward 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 50, 23 pursued another cycle of IVF after antibiotic treatment. The 23 patients' mean age was 38.7 ( $\pm 4.5$  SD) years. The mean ages for the Malizia and Elizur studies were 35.8 ( $\pm 4.7$ ) and 32.7 ( $\pm 5.9$ ) 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)

Table 1. Comparing Two Historical Controls for Chance of Delivery (Relative Risk) after Antibiotic Therapy. Study II.

No. 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
Totals	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 $P < 0.0001$			

Similar statistical calculations were performed after combining the data from our first study (Study I, 52 treated couples, mean age 38.1 ( $\pm 4.1$  SD) years, Reference 24) with the recent study (Study II, 63 treated couples), on a total of 105 cases. All of these couples experienced one or multiple IVF failures before undergoing antibiotic therapy. Again, only cases with fewer than 5 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, 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 to the patients in Malizia's report, those receiving antibiotics were 3.6 times more likely to deliver on the current cycle than the controls ( $P < 0.0001$ ,  $RR = 3.6$ ,  $95\% \text{ CI: } 3.0 - 4.4$ ). When compared to the controls in the Elizur publication the delivery rate was 6.1 times greater ( $P < 0.0001$ ,  $RR = 6.1$ ,  $95\% \text{ CI: } 4.9 - 7.7$ ).

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. Prior Failures	Antibiotic (Toth) Delivery		Malizia Delivery		Antibiotic (Toth) Delivery		Elizur 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
Totals	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			

*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 (Table 5).

<b>Table of Pregnancy Type by Study</b>			
<b>Pregnancy Type</b>	<b>Study</b>		
<b>Frequency Col Pct</b>	<b>Study I</b>	<b>Study II</b>	<b>Total</b>
<b>No Deliveries</b>	29 44.23	28 55.55	57
<b>Twins</b>	4 7.69	7 11.11	11
<b>Spontaneous Singleton</b>	3 5.77	12 19.05	15
<b>Singleton 1st IVF post antibiotic therapy</b>	14 26.92	16 25.40	30
<b>Singleton 2nd IVF post antibiotic therapy</b>	2 3.85	0 0.00	2
<b>Total Number of couples</b>	52	63	115

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



Study	N		Variable	Label	N	Mean	Median	Std Dev	Lower Quartile	Upper Quartile
	Obs									
Study 1	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 2	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

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

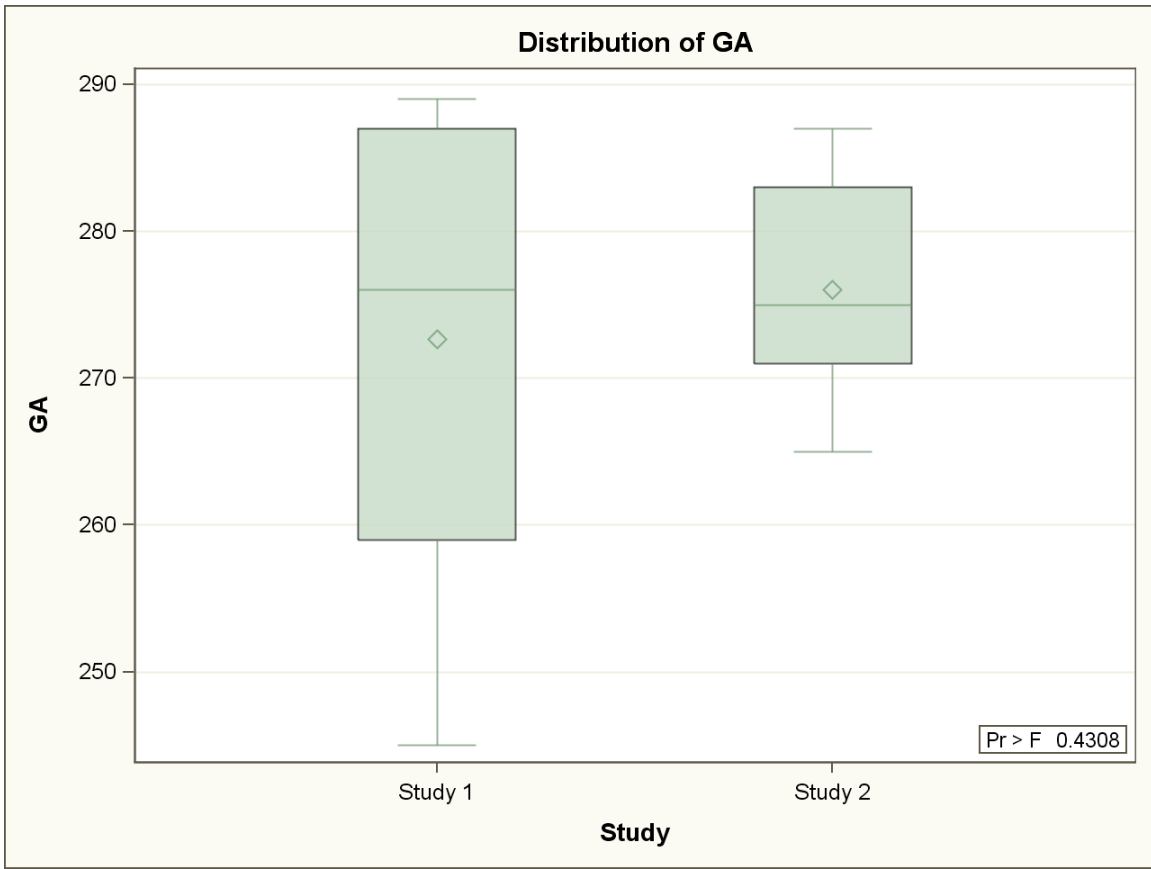


Table 5. Comparison of gestational age (GA in days) at delivery, Study I and Study II

# 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 preconceptionally 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, Ref.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 male factor in infertility and in 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 PCR, rather than a reinfection. We are aware of the emergence of multi- drug 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 males with clinical, laboratory or sonographic evidence of chronic prostatitis were given five direct trans rectal antibiotic injections using the described cocktail. Neither the pretreatment Chlamydia positive culture status nor the total number of pretreatment bacterial isolates were predictors of a successful pregnancy. The small sample size however prevents a significant conclusion. Clearly, more detailed microbiological studies are indicated. In general, IVF pregnancies have an increased risk of developing pregnancy-associated complications, such as bleeding, preeclampsia, 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 extreme low or low birth weight and suffer from intrauterine growth retardation and congenital malformations (39,40,41,42). In our Study II, none of the patients delivered prematurely and none of the babies were of small birth weight and there were no intrauterine growth retarded newborns. Our series lacks the complicated pregnancies and the deliveries of sickly children. Our

singleton newborns did not require any NICU days in hospitals. 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 and most likely this is the same infection that has rendered the woman infertile and in need of the IVF procedure to start with.

It is tempting to postulate that the common observation that IVF pregnancies yield infants with an increased number of medical problems that result in higher health care costs in the future (42) could be due to the mothers' infected uterine

environment. Thus preventive medicine in the form of antibiotic therapy prior to the first IVF attempt could contribute to a reduction in future health costs.

We conclude therefore that in a large number of IVF failures, a direct uterine and/or seminal fluid infection plays a role. In both of our series, following antibiotic therapy, up to age 43 there was no significant decline in the woman's chance of achieving a subsequent pregnancy after previously failed IVF cycles, suggesting that in a number of these cases it could be a low-grade bacterial contamination of the female and male reproductive canals that renders women of all ages sub fertile and has a cumulative disproportionate effect on women above forty. We concur with other investigators who find Chlamydia to be a significant pathogen.



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