

## A challenge to the concept that the use of calcium channel blockers causes reversible male infertility

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The objective of this study was retrospectively to evaluate both in-vitro fertilization (IVF) and non-IVF cycles in which the male partner had been taking calcium channel blockers, either to confirm or refute previous data from another centre, suggesting that these drugs cause a severe but reversible subfertility problem in the male. These drugs were found to inhibit expression of mannose-ligand binding receptors, thus preventing spermatozoa from attaching to the zona pellucida; they were postulated to cause failed fertilization based on one case having this defect, in whom a return to normal was achieved after stopping the drug. However, the couple did not undergo a cycle with IVF to see if fertilization now occurred. The data presented here demonstrated fertilization in all patients having IVF who were taking calcium channel blockers. The subsequent pregnancy rate per transfer was 17.4%. Also, five out of 11 (45.4%) non-IVF patients conceived after correction of various female factors. Failure of the other six patients to conceive could be explained by other confounding factors, especially oligoasthenozoospermia. Taking into consideration other data suggesting poor fertilization when this mannose-ligand binding receptor abnormality was demonstrated, we propose the possibility that this defect, when not associated with calcium channel blockers, may be associated with some other cryptic factor that causes poor fertilization. According to our hypothesis, calcium channel blockers might cause the problem in mannose expression but also adversely affect some other factor that is deficient when non-drug related abnormalities in mannose-ligand binding expression are found.

**Key words:** calcium channel blockers/failed fertilization/IVF/mannose-ligand binding receptors

### Introduction

It has been reported that normozoospermic males who fail to fertilize oocytes following in-vitro fertilization (IVF) have a defect in the ability to increase the percentage of spermatozoa with plasma membrane mannose-ligand receptor expression over the acrosome and post-acrosomal regions

of the sperm head and the percentage of spermatozoa exhibiting spontaneous and mannose-induced acrosome reactions following incubation of spermatozoa under standard capacitating conditions (Benoff *et al.*, 1993a,b). Benoff *et al.* (1994) described a case of failed fertilization associated with decreased mannose-ligand receptors. The lack of expression of head-directed mannose-ligand receptors at high frequency and the failure to undergo spontaneous acrosome loss was attributed to the use of calcium channel blockers, since switching the male partner to an angiotensin-converting inhibitor completely reversed these abnormalities; in fact, sperm characteristics returned to those of fertile controls (Benoff *et al.*, 1994). Similarly, nine additional males taking calcium channel blockers had abnormalities in mannose-ligand binding receptor expression, and in three who changed to alternative antihypertensive therapy, there were also changes in semen parameters similar to those occurring in fertile controls (Benoff *et al.*, 1994).

Benoff *et al.* (1994) assumed that the change in expression of mannose-ligand binding receptors related to the use of calcium channel blockers was the cause of the failed fertilization in the signal case. However, they failed to prove Koch's postulates by demonstrating that the reversal of the abnormal semen parameters to those of fertile controls after stopping the calcium channel blocker was associated with a return to normal fertilization. Furthermore, they never demonstrated that the nine additional males demonstrating abnormalities in expression of mannose-ligand binding receptors and in spontaneous and mannose-induced acrosomal loss actually failed to fertilize oocytes following IVF.

The study presented here retrospectively evaluated fertilization rates and pregnancy outcome of couples undergoing IVF or non-IVF cycles where the male partner had been treated with calcium channel blockers for at least 6 months prior to the start of infertility procedures and continued the therapy during the entire course of infertility treatment.

### Materials and methods

#### Study 1

A retrospective analysis was performed on all IVF cycles from 1990 to 1994 to identify cycles where the male partner had been using calcium channel blockers. The identification was initially through computer files but all cases were confirmed by subsequent telephone interview to be sure the medication was actually taken during the retrieval. If the patient was uncertain, their case would be eliminated. All 11 couples confirmed that the male partner was taking his calcium channel blocker medication for at least 6 months prior to and including the day of oocyte retrieval. The class of drugs included: 1,4-

**Table I.** Fertilization and pregnancy rates in couples undergoing in-vitro fertilization (IVF) where the male partner was taking calcium channel blockers

Drug	Couple no.	Cycle no.	No. oocytes retrieved	No. oocytes fertilized	% fertilization	Pregnant
Verapamil	1	1	8	8	100	yes
Verapamil	2	1	3	2	67	no
Cardizem	3	1	5	3	60	no
Verapamil	4	1	34	22	65	no
Cardizem	5	1	28	22	74	yes
Verapamil	6	1	4	4	100	no
Verapamil	6	2	10	9	91	no
Verapamil	7	1	3	2	67	no
Verapamil	7	2	3	3	100	no
Procardia	8	1	16	13	81	no
Procardia	8	2	18	9	50	no
Procardia	8	3	20	15	75	yes
Verapamil	9	1	11	7	64	no
Verapamil	9	2	9	3	33	no
Verapamil	9	3	9	3	33	yes
Cardizem	10	1	8	8	100	no
Cardizem	10	2	5	3	60	no
Cardizem	10	3	6	5	83	no
Cardizem	10	4	8	4	50	no
Verapamil	11	1	6	3	50	no
Verapamil	11	2	12	7	58	no
Verapamil	11	3	6	4	67	no
Verapamil	11	4	6	3	40	no
Mean			10.35	7.04	68.17	
SD			8.01	5.85	20.85	
Median			8.00	4.00	67.00	
Minimum			3	3	33	
Maximum			34	22	100	

dihydropyridines (procardia), benzothiazepines (cardizem) and phenylalkylamines (verapamil).

The 11 couples underwent a total of 23 IVF-embryo transfer cycles. All cycles used standard IVF procedures (no intracytoplasmic sperm injection) and oocytes were inseminated with 10 000 motile morphologically normal spermatozoa.

The ovarian stimulation regimen for all cycles used leuprolide acetate (TAP Pharmaceuticals, Inc., Deerfield, IL, USA) 1 mg s.c. beginning 1 week after ovulation for at least 10 days until serum oestradiol was <50 ng/ml (SI units = 183.5 pmol/l) and serum progesterone <1 ng/ml (SI units = 3.18 pmol/l) when the dosage of leuprolide acetate was reduced to 0.5 mg s.c. and human menopausal gonadotrophin (HMG; Perganol, Serono, Randolph, MA, USA) was started at 300 IU/day i.m. in two divided doses. Oocyte retrieval occurred 36 h after 10 000 IU i.m. of human chorionic gonadotrophin (HCG; Profasi, Serono) and embryo transfer occurred 3 days later.

### Study 2

There were 11 couples attempting conception without the use of assisted reproductive technology. At the time of the initial semen analysis, a drug interview was obtained and only those patients who confirmed that they were on the medication for at least 6 months were included in the study. Each case was treated on an individual basis following testing to determine the aetiology of infertility. Thus, anovulatory patients were treated with follicle maturing drugs, e.g. clomiphene citrate or gonadotrophins, cervical and male factor infertility was treated with intrauterine insemination, and luteal phase defects with supplemental progesterone in the luteal phase. Pregnancy rates during the first 6 months of infertility treatment were then recorded.

## Results

### Study 1

Seven men used verapamil (Calan, Searle, Chicago IL and Verelan, Lederle, Pearl River, NY, USA), three used cardizem (Hoechst Marion Roussel, Kansas City, MO, USA), and one took procardia Pfizer, New York, NY, USA). There were a total of 23 IVF cycles. Two couples had four IVF cycles, two had three cycles, two had two cycles and five couples had only one IVF cycle each. The fertilization rates for each cycle are shown in Table I. The type of calcium channel blocker and indication of achievement of pregnancy is also indicated in Table I. The mean fertilization rate was comparable with couples in our programme where the male partner was not taking calcium channel blockers. Four of the 11 couples achieved a pregnancy (36.3% per patient, 17.4% per cycle).

### Study 2

Nine men used verapamil, one used cardizem, and one used procardia. Pregnancies occurred in five of the 11 (45.4%) patients. The median and mean ages were 33 and  $33.8 \pm 4.15$  years for those achieving pregnancy compared with 35 and  $36.3 \pm 5.68$  for non-conceivers. Oligozoospermia and/or asthenozoospermia was present in five of the six males not achieving a pregnancy. Pregnancies occurred in the female partners of three of nine (33.3%) males taking verapamil, one of one (100%) male using cardizem, and also the only male treated with procardia (100%).

## Discussion

The previous demonstration of an association of abnormal expression of mannose-ligand binding receptors and poor fertilization does not necessarily prove cause and effect (Benoff *et al.*, 1993b). The possibility exists that when this abnormal sperm function occurs in men not taking calcium channel blockers, it may be associated with some other as yet undiscovered abnormal sperm function that is the main factor causing the reduced fertility potential of the spermatozoa. Thus, our data could be consistent with the hypothesis that calcium channel blockers can inhibit mannose-ligand receptor expression on sperm heads after capacitation but do not suppress some other cryptic factor needed for fertilization that usually accompanies this defect when not related to use of calcium channel blockers.

Recently, the Cornell group presented an abstract at the 12th Annual Meeting of the ESHRE in Maastricht (1996), demonstrating that four of five patients achieved a pregnancy after the male partner stopped taking calcium channel blockers (Hershlag *et al.*, 1996). In none of these cases had it been demonstrated that the couples failed to fertilize while on calcium channel blockers. Three of the couples had specific treatments rendered to the wives for female factors. The fact that two of the patients were able to achieve fertilization with IVF, after being taken off the calcium channel blockers, does not have much significance in view of never having previously demonstrated failed fertilization, especially since we demonstrated that all 11 of our couples in study 1 taking calcium channel blockers had adequate fertilization. The other three couples all achieved pregnancies after the men stopped using calcium channel blockers (Hershlag *et al.*, 1996), but this could also be fortuitous since our study 2 found pregnancies occurring in five of 11 (45.4%) treated couples where the male was still taking calcium channel blockers during the infertility therapy. Furthermore, there was moderate oligoasthenozoospermia rather than unexplained infertility plus advanced age of the female partner to explain why the other 55% did not conceive in our study.

The possibility exists that the nifedipine used by the majority of men in the Cornell study has more of an adverse effect on sperm function than does verapamil, which was used by the majority of men in the study presented herein. For the IVF group only one of the 11 men took a 1,4-dihydropyridine class drug similar to nifedipine and that was procordia; in contrast, seven of the 11 took verapamil in the present IVF study. Data have previously been presented suggesting that verapamil may increase sperm motility and accelerate acrosome reaction (Roldan *et al.*, 1987). The one patient in our IVF study taking procordia had three cycles of IVF and the fertilization rates were 81, 50, and 75% respectively; a pregnancy was achieved. For the non-IVF group, the large majority also were treated with verapamil (nine of 11) but both remaining patients (one taking procordia and one using cardizem) achieved pregnancies.

Another confounding variable that could exist between the Cornell study and the study presented here is possibly different patient demography. Afro-American patients, for example, may be more sensitive to calcium antagonists than Caucasian

patients. In the present study, 10 of the 11 IVF patients were Caucasian, as were 10 of the 11 non-IVF cases. The Cornell study demography is not known.

The Cornell group found that the concentration of calcium channel blockers needed to demonstrate in-vitro inhibitory effects on parameters of sperm fertilizing potential were two to four orders of magnitude below the average estimated concentration of these drugs in the general circulation after long-term administration (Benoff *et al.*, 1994). The possibility exists, however, that these drugs do not penetrate the testis or get into the seminal plasma. However, the in-vivo studies by this group demonstrating the mannose-ligand receptor expression and acrosome abnormalities in males taking therapeutic dosages of calcium channel antagonists, which are reversible when switching to other antihypertensive drugs, certainly suggest that these drugs penetrate into the testes and/or seminal plasma.

There would be difficulty in finding a sufficient number of cases where men are taking calcium channel blockers in any one IVF centre to perform a randomized prospective study. It is hoped that this retrospective review will stimulate other centres to perform a similar evaluation and perhaps more can be learned by correspondence between multiple centres. The data presented here certainly do not support the prior conclusion by the Cornell group that the use of any calcium channel antagonist by the male partner is associated with inevitable failed fertilization and infertility.

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