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Effectiveness of pentoxifylline in semen preparation for intrauterine insemination

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Pentoxifylline, an inhibitor of cAMP phosphodiesterase activity, favours intracellular cAMP concentration increase. In-vitro treatment of semen with pentoxifylline leads to marked augmentation of sperm motility, enhancement of acrosome reaction, increase of sperm penetration into zona-free hamster oocytes, and protection of the sperm plasma membrane. Such properties indicate that the drug may be a useful tool for semen preparation in assisted reproduction, but its real effectiveness in improving fertilization rates is still uncertain, mainly in association with intrauterine insemination (IUI). Theoretically sperm motility should play an extremely important role for positive results in IUI. Therefore a retrospective clinical trial was planned in order to evaluate whether addition of pentoxifylline to the previously standardized in-vitro treatment of semen had improved the percentage of pregnancies after homologous IUI. The study involved 55 sterile couples (33 classified infertile for male factor and 22 for other factors) who underwent a total of 150 cycles of homologous IUI: 101 for male factor infertility and 49 for other factors (anovulation $n = 26$, endometriosis $n = 2$, idiopathic $n = 21$). Out of the 101 cycles performed for male factor infertility, 61 underwent the standard preparation of semen and were followed by seven pregnancies (pregnancy rate = 11.5%) while 40 had a semen preparation with pentoxifylline addition and were followed by 11 pregnancies (pregnancy rate = 27.5%) with a significant difference between the two procedures ($P < 0.05$). Out of the 49 cycles carried out for factors different from male infertility, 10 underwent the standard preparation of semen and were followed by two pregnancies (pregnancy rate = 20.0%), while 39 had pentoxifylline addition and were followed by nine pregnancies (pregnancy rate = 23.1%). The difference between the two groups was not significant. Abortions and malformations were equally distributed in the standard treatment and in the pentoxifylline group.

Key words: intrauterine insemination (IUI)/pentoxifylline/semen preparation

Introduction

Pentoxifylline, like other methylxantine derivatives, acts as an inhibitor of cyclic 3′,5′-adenosine monophosphate (cAMP) phosphodiesterase activity (Schoenfeld *et al.*, 1975; Aitken *et al.*, 1986). As a result of this effect, intracellular cAMP concentration increases leading to a marked augmentation of sperm motility (Garbers *et al.*, 1971; Tash and Means, 1982; Ward and Clissold, 1987). In human spermatozoa a positive correlation between adenylyl cyclase activity, cAMP content, and sperm motility has been shown (Ishikawa *et al.*, 1989). As regards motility, some authors have found that pentoxifylline leads to a significant increase of the number of motile spermatozoa both in normozoospermic and asthenozoospermic samples (McKinney *et al.*, 1994), as well as in specimens obtained by electroejaculation (Sikka and Hellstrom, 1991). On the other hand, it has also been reported (Aparicio *et al.*, 1980; Yovich *et al.*, 1990; Tesarik *et al.*, 1992a) that pentoxifylline does not increase the number of motile spermatozoa, although it improves sperm motion in preselected subpopulations of spermatozoa both in asthenozoospermic (Tesarik *et al.*, 1992a) and normozoospermic samples (Rees *et al.*, 1990; Lewis *et al.*, 1993). Pentoxifylline has also been shown to enhance acrosome reaction (Cummins *et al.*, 1991), to increase sperm penetration into zona-free hamster oocyte (Lambert *et al.*, 1992), and moreover to protect the sperm plasma membrane through an antioxidant action (Gavella *et al.*, 1991) by removing peroxides formed from free radicals (Yovich, 1993).

Although pentoxifylline is gaining popularity as a tool to optimize sperm preparation for assisted reproduction, its real effectiveness in improving fertilization rates is still uncertain, mainly in association with intrauterine insemination (IUI). In fact, most of the reports deal with in-vitro fecundation (Yovich *et al.*, 1990) and little is known about the effectiveness of pentoxifylline in IUI. Sperm motility is thought to play an extremely important role for positive results in IUI, and even more important is the ability to maintain motility over time as insemination and ovulation may not coincide exactly (Denil *et al.*, 1992). In fact, the peculiarity of this technique is that the rendezvous of oocyte and spermatozoon does not occur in the artificial milieu of a Petri dish, but in almost natural conditions, that are out of laboratory control.

Since pentoxifylline treatment significantly increases the sperm capacity to undergo the acrosome reaction in response to both natural and artificial stimuli (Tesarik *et al.*, 1992b), there is a reasonable concern about possible untimely occurrence of extensive acrosome reactions. Therefore a retrospective clinical trial was planned in order to evaluate whether addition of pentoxifylline to the previously standardized in-vitro treatment of semen improves the percentage of pregnancies after homologous IUI.

Materials and methods

Patients

The study involved 55 sterile couples, 33 of whom were classified infertile for male factor and 22 for other factors, who underwent a total of 150 cycles of homologous IUI at the Reproductive Medicine Unit of the University of Ferrara, Italy during 8 months. In the middle of this period the standard method for the preparation of semen was modified at one single step, that is the supplementation of pentoxifylline. The purpose of the trial was to compare the pregnancy rate in the first 4 months, when semen was prepared in the absence of pentoxifylline, with the pregnancy rate in the last 4 months, when pentoxifylline addition became a routine step in semen preparation. Over this period some patients were treated only with or without pentoxifylline supplementation, but 12 of them, who were under treatment just when the preparation of spermatozoa was modified, underwent both laboratory procedures in different cycles. The significance of pentoxifylline addition during the preparation of semen for IUI was therefore better expressed by pregnancy rate per cycle than per patient. Among the 33 infertile men, 21 underwent the standard treatment without pentoxifylline and 24 were subjected to pentoxifylline addition. Out of the 21, 16 were asthenozoospermic with average cell number = 85×10^6/ml (25–200), rapid progressive motility = 25% (1–45), sluggish progressive motility = 9% (1–29), and rapid progressive motility after treatment = 53.9% (20–85); five were oligoasthenozoospermic with average cell number = 15×10^6/ml (10–20), rapid progressive motility = 32.8% (15–45), sluggish progressive motility = 2% (0–10), and rapid progressive motility after treatment = 54.4% (27–78). Out of the 24 patients whose semen was treated with pentoxifylline, 17 were asthenozoospermic with average cell number = 62×10^6/ml (30–15), rapid progressive motility = 23.6% (0–40), sluggish progressive motility = 11.9% (0–37), and rapid progressive motility after treatment = 63.7% (35–90); seven were oligoasthenozoospermic with average cell number = 15×10^6/ml (10–20), rapid progressive motility = 18.5% (0–38), sluggish progressive motility = 13.8% (0–50), and rapid progressive motility after treatment = 64.7% (35–100). As to morphology, no sample was teratozoospermic.

Semen collection and preparation

All subjects were requested to abstain from any sexual activity for 3–4 days before semen collection. Semen specimens were obtained by masturbation and collected in a dry, wide-mouthed, sterile, plastic container. The ejaculate was allowed to liquefy at room temperature and was then analysed using a Makler counting chamber according to the World Health Organization guidelines (WHO, 1992).

Standard treatment

Specimens for insemination were prepared according to the instructions of the Bourn–Hallam Group (Elder *et al.*, 1990) with slight modifications.

Spermatozoa were separated through a discontinuous Percoll gradient. Isotonic Percoll was prepared by diluting 45 ml of Percoll (Pharmacia, Uppsala, Sweden) with 5 ml 10× concentrated EBSS (Life Technologies Ltd, Paisley, Scotland), 4.5 ml 5% human albumin, 2.4 mg sodium lactate, 1 ml 1 M Hepes (Life Technologies Ltd, Paisley, Scotland). Gradients were made with 2.5 ml 80% Percoll (4 ml isotonic Percoll in 1 ml EBSS 1X) overlayered by 2.5 ml 40% Percoll (2 ml isotonic Percoll in 3 ml EBSS 1X). In the last step 2.0 ml of freshly collected semen were layered on top of 40% Percoll and then centrifuged at 600 *g* for 20 min to obtain a pellet of functionally normal spermatozoa.

Table I. Pregnancy rates per cycle (%) in couples affected by male factor/other factor infertility undergoing intrauterine insemination using semen prepared with a standard treatment or after addition of pentoxifylline (PTF)

	Male factor	Other factors	Overall
Standard treatment	11.5 (7/61)	20.0 (2/10)	12.7 (9/71)
PTF treatment	27.5 (11/40)	23.1 (9/39)	25.3 (20/79)
P-value	$P < 0.05$	NS	NS

NS = not significant.

Pentoxifylline treatment

Pentoxifylline (Sigma, St Louis, MO, USA) was dissolved (1 mg/ml) in 1X Ham's F-10 Medium (ICM Biomedicals, Costa Mesa, CA, USA). An aliquot of 1 ml of freshly collected semen was mixed with 1 ml of pentoxifylline solution and incubated at 37°C for 10 min. At the end of the incubation the sample was layered on a Percoll gradient and centrifuged as previously described.

Whatever the treatment, the pellet was resuspended in 0.2 ml of Irvine washing medium (Irvine Scientific, Santa Ana, CA, USA), re-evaluated, and inseminated with a Kremer–Delafontaine catheter (Laboratoire CCD, Paris, France) 36 h after human chorionic gonadotrophin (HCG; 10 000 IU) injection.

Statistical analysis

χ^2 test was applied as appropriate for comparison of percentages. A P value < 0.05 was taken to be indicative of a statistically significant difference.

Results

In 8 months 150 cycles of IUI were performed, 101 for male factor infertility, 49 for other factors: anovulation ($n = 26$), endometriosis ($n = 2$), idiopathic ($n = 21$).

As shown in Table I, 61 out of the 101 cycles performed for male factor infertility underwent the standard preparation of semen and were followed by seven pregnancies (pregnancy rate = 11.5%), while 40 had a semen preparation with pentoxifylline addition and were followed by 11 pregnancies (pregnancy rate = 27.5%), with a significant difference between the two methods ($P < 0.05$).

Out of the 49 cycles carried out for factors different from male infertility, 10 underwent the standard preparation of semen and were followed by two pregnancies (pregnancy rate = 20.0%), while 39 had pentoxifylline addition and were followed by nine pregnancies (pregnancy rate = 23.1%). The difference between the two groups was not significant.

Abortions were equally distributed in the standard treatment group ($n = 3$) and in the pentoxifylline group ($n = 4$).

A case of angioma in one twin and multiple anomalies of the external ear in two of a triplet (resembling a similar defect in their mother) were the only malformations appearing, and they were found in the standard treatment group.

Discussion

There is no doubt that pentoxifylline improves the motility of spermatozoa, but several conflicting reports concerning the quality of motility have been published. Pentoxifylline has been found (Yovich *et al.*, 1988; Rees *et al.*, 1990; Lewis

et al., 1993) to have no effect on the number of progressively motile spermatozoa in normozoospermic samples but to increase the number of progressively motile spermatozoa (Yovich *et al.*, 1988; Sikka and Hellstrom, 1991) and to improve percentage motility (McKinney *et al.*, 1994) in asthenozoospermic samples. Marrama *et al.* (1985) obtained an increase in percentage motility in idiopathic oligoasthenozoospermic subjects with oral administration of the drug.

Other authors suggest that pentoxifylline effect is limited to an increase in sperm velocity in asthenozoospermic samples (Shen *et al.*, 1991; Kholkute *et al.*, 1992) and that it has no effect on the VSL (straight line velocity), while having a dramatic and consistent effect on VCL (curvilinear velocity) (Tesarik *et al.*, 1992a; Lewis *et al.*, 1993). In contrast, Sikka and Hellstrom (1991) demonstrated an increase of sperm velocity, VSL, and mean linearity. It has also been reported that linearity does not increase in parallel with an increase in VSL (Bongso *et al.*, 1989), that pentoxifylline induces forward acceleration, augmentation of lateral head excursions, and intensification of flagellar beat (Tesarik *et al.*, 1992a) and also that it significantly improves sperm forward progressive motility (Dimitriadou *et al.*, 1995).

These apparently conflicting and not easily comparable results may be ascribed to different doses (Paul *et al.*, 1995) or to different time exposure to the drug (Lewis *et al.*, 1993; Moohan *et al.*, 1993). Particularly important in determining the quality of results is in fact the time exposure as well as the criteria in selecting and/or preparing the samples or the subpopulations for the trial (McKinney *et al.*, 1994). Moreover, individual responses to the drug have recently been demonstrated (Moohan *et al.*, 1993).

In any event, apart from the real effect induced by pentoxifylline, it has been reported that in IUI sperm velocities are significantly lower in conceptual than in non-conceptual cycles (Tucker *et al.*, 1991). Similarly spermatozoa with reduced VSLs have a higher fertilization rate of zona-free hamster egg (Cohen *et al.*, 1982).

What is even more uncertain about pentoxifylline is the demonstration of its role in pregnancy rate improvement when patients are inseminated with pentoxifylline treated semen. Recently it has been shown that in an IVF programme the fertilization success and pregnancy rate are unrelated to pretreatment of semen with the drug (Dimitriadou *et al.*, 1995) and that there is no therapeutic advantage to using pentoxifylline in IVF for male factor infertility (Tournaye *et al.*, 1993).

One might reasonably expect that the advantages of using pentoxifylline in IUI are even less than in IVF. In fact, the drug seems to sensitize the spermatozoa to undergo acrosome reaction for both natural and artificial stimuli (Tesarik *et al.*, 1992b). For this reason the potential beneficial action of pentoxifylline on sperm motility could be undermined by the adverse effect of inducing a premature massive acrosome reaction of the prepared spermatozoa as soon as they are inseminated in the uterine cavity or anyhow before their contact with the oocyte.

We have shown that the treatment of oligoasthenozoospermic semen with pentoxifylline leads to a significant increase in the pregnancy rate after IUI, while treatment of normozoospermic samples does not cause any significant increase. Therefore spermatozoa which have no motility defect do not benefit from pentoxifylline treatment, in agreement with those authors who have found that pentoxifylline has no effect on the number of progressively motile spermatozoa in normozoospermic samples (Yovich *et al.*, 1988; Rees *et al.*, 1990; Lewis *et al.*, 1993).

Unfortunately our clinical trial does not allow any direct comparison of the relationship between the effect of pentoxifylline on the pregnancy rate and its action on the acrosome reaction; in fact only the final combined effect of increased capacity to undergo acrosome reaction plus increased motility can be detected. Our results show that in normozoospermic partners, in which sperm motility improvement does not play any role, the pregnancy rate is not reduced, but even slightly increased, by pentoxifylline if compared with untreated patients. This suggests that the potential action of the drug on the acrosome reactivity does not negatively affect the fertilizing ability of treated spermatozoa.

An impairment of early zygote development using standard concentrations of pentoxifylline was demonstrated in an experimental IVF model in mice (Dimitriadou *et al.*, 1995), suggesting that after drug administration spermatozoa should be carefully washed out before in-vitro insemination. Our standard technique of semen preparation does not include washing steps after pentoxifylline treatment, though leading to positive results with IUI. At present it cannot be stated whether spermatozoa become free of pentoxifylline after centrifugation through Percoll gradients and whether possible remnants of the drug are completely removed during their migration in the female genital tract.

After the successful fertilization of a human oocyte by microinjection of a testicular spermatozoon (Schoysman *et al.*, 1993), almost all problems related to male infertility seemed to be resolved. Tournaye *et al.* (1993), after demonstrating the uselessness of pentoxifylline, suggested renouncing IVF for treatment of male factor infertility in favour of micromanipulation techniques. Our experience enables us to suggest that couples affected by male factor sterility should be allowed a chance with IUI: in our clinical practice three attempts are usually performed before an oligoasthenozoospermic patient is recommended to undergo the much more expensive microinjection treatments. The two poorest treated samples resulting in a subsequent successful pregnancy were characterized by sperm concentrations of 13×10^6/ml and 30×10^6/ml with rapid progressive motilities of 23 and 10% respectively. It is our opinion that patients whose ejaculate reaches at least this minimal threshold should be considered for IUI.

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