THERAPEUTICAL USE OF PENTOXIFYLLINE IN DISTURBED MALE FERTILITY

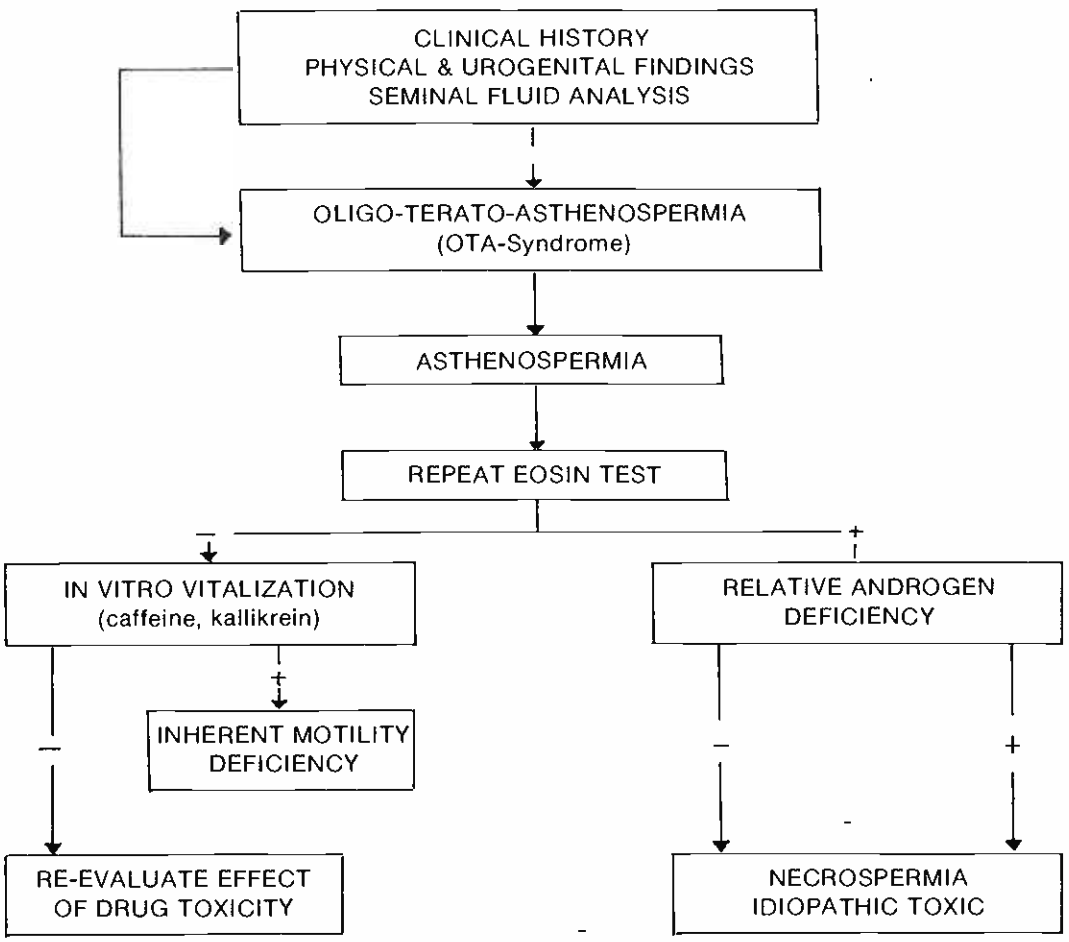
Dr. Nestor J. Aparicio

Grupo de Estudios en Fertilidad y Endocrinologia de Rosario Corodoba 1764. Rosario, Santa Fe, Argentina.

The incidence of human sterility (that is couples unable to conceive a child) is approximately 13% in Argentina. At least 40% of infertile couples have a male partner with problems that lead to reversible or irreversible infertility. The evaluation of the fertility state of a man is difficult since not only the concentration of spermatozoa per ml should be taken into consideration but also the quality of spermatozoa.

At present there is no uniform concept on the figures of a normal spermogram. In agreement with several authors we consider that a normal spermogram should have more than 60% of normal oval forms, less than 6% of tapering forms, less than 0.5% of immature forms and less than 8.6% of amorphous forms (Glezerman, M., et al., 1978). On the other hand, not less than 30% of the spermatozoa should be forwardly progressive, that means spermatozoa with straight and fast movement. Concentration should be higher than 20.0×10^5 sperm per ml. Patients with spermograms showing low motility in at least three samples are considered to be asthenospermic. Asthenospermia is an entity that could be determined by several factors such as alterations of the hypothalamic pituitary gonadal axis, infective and/or inflammatory processes at the genital tract, urologic diseases, immunologic diseases, genetic alterations and the entity which is called "inherent motility deficiency" (Glezerman, M., et al, 1978). Figure 1 shows the approach to the diagnosis of some causes of asthenospermia. The main problem related to asthenospermia is that therapeutic failures are really the rule. Due to this fact several attempts have been performed to obtain successful sperm vitalizing therapy. Some authors have reported that methylxanthines may increase the percentage, quality and duration of activity of ejaculated human spermatozoa (Schoenfeld C., et al, 1975; Haesungsharern, A., Chulavanatal, M. 1973; Turner, E.A. de., et al., 1978. Since methylxanthines are agents which inhibit phosphodiesterase, the reported effects could be due to an increase in the concentrations of cyclic adenosine 3,5' monophosphate (cAMP) and cyclic guanosine 3,5' monophosphate within spermatozoa, probably influencing the oxidative metabolism in these cells (Schoenfeld C., et al, 1975, Hicks, J.J., et al, 1972). Pentoxifylline is a new agent which has a proved phosphodiesterase inhibitory action and a long-lasting effect and a higher hydrosolubility than other agents of the methylxanthine group such as caffeine or theophylline (Stefanovich, V., 1973; Popendiken K., et al, 1971).

Figure 1 Male Infertility



On these bases, the following investigations were performed with pentoxifylline. 1) Approach to the action of pentoxifylline on human sperm motility in comparison to other agents. 2) Dose response effect of pentoxifylline on the motility of normal human spermatozoa. 3) Effect of pentoxifylline on spermatozoa of patients with asthenospermia. 4) Use of pentoxifylline in homologous artificial insemination with semen samples of patients with asthenospermia. 5) Treatment with pentoxifylline by oral administration in patients with asthenospermia. 6) Artificial insemination in cows using pentoxifylline.

1) Approach to the action of pentoxifylline on human sperm motility in comparison to other agents.

Semen samples were obtained by masturbation from 18 normal men volunteers (aged 18 to 42 years). All samples had volumes greater than 3 ml with at least 20 x 10^6 spermatozoa per ml. Immediately after liquefaction (approximately 30 minutes after collection) each sample was mixed well and seven 0.3 ml aliquots were taken. One aliquot was used as control; another aliquot was combined with Mann-fructose fluid (pH 7,4) in the proportion of one part to five parts of semen. The other aliquots were respectively combined with caffeine, pentoxifylline (BL 191), dibutyryl cAMP, propranolol and propranolol + dibutyryl cAMP (all of them in 0,06 ml of Mann-fructose flud) to give a final concentration of 0,6mM. The aliquots were incubated at 37±C and well mixed before fractions were removed and examined by light microscopy at 30 minutes and 1, 2 and 4 hours after collection (Mac Leod J., 1965). Motility was graded on a 4-point scale as follows: 1) no motion; 2) sperm moving with no forward progression (*in situ* motile spermatozoa); 3) sperm moving with a slow forward motion (slowly progressive spermatozoa); 4) sperm moving rapidly or with high speed in almost straight line (forwardly progressive spermatozoa). The number of dead spermatozoa was determined by using an eosin-nigrosin technique (Dougherty, K.A., et al, 1975). Results were expressed in percentage referred to the total number of observed spermatozoa per aliquot and time of observation according to the following categories: forwardly progressive spermatozoa, slowly progressive spermatozoa, *in situ* motile spermatozoa, live and non-motile spermatozoa and dead spermatozoa (Turner, E.A., et al, 1978). The number of live and non-motile spermatozoa was calculated by subtracting the number of dead spermatozoa from the total number of non-motile spermatozoa. Results were evaluated by using analysis of variance to compare results of the different groups at each time of observation.

Figure 2 shows that control semen (group S) experienced a progressive decrease of the percentage of forwardly progressive spermatozoa, reaching a minimum at the 4th hour after collection. The addition of Mann-fructose fluid to the semen (group M) resulted in significantly lower percentages of forwardly progressive spermatozoa as compared with controls at the first hour ($p < 0.001$) and 2nd hour ($p < 0.001$) after collection; the percentage of dead spermatozoa was higher in this group than in the control semen ($p < 0.001$) at the 4th hour after collection. Semen combined with Mann-fructose fluid + caffeine (group C) behaved in a manner similar to control semen. The percentage of forwardly progressive spermatozoa in semen combined with Mann-fructose fluid + pentoxifylline (group B) was at the 4th hour significantly higher than that of the control group. This percentage was also higher than that obtained with caffeine ($p < 0,05$). The percentage of dead spermatozoa at the 4th hour and in the group treated with pentoxifylline was significantly lower than that obtained with Mann-fructose fluid alone and with Mann-fructose fluid with caffeine. The aliquots combined with Mann-fructose fluid + cAMP (group A) showed results similar to the ones obtained in the group treated with pentoxifylline.

In semen combined with Mann-fructose fluid + propranolol (group P) or with propranolol + cAMP (group PA) no forwardly progressive spermatozoa were observed at any time.

From these results it can be concluded: a) observation of control semen samples showed the expected time dependent decrease in motility; b) the addition of Mann-fructose fluid to the semen resulted in a decrease of motility and an increment in the percentage of dead spermatozoa; c) the addition of caffeine seemed to neutralize the deleterious effects of Mann-fructose fluid; d) pentoxifylline and cAMP resulted not only in a neutralization of the deleterious effects of Mann-fructose fluid but also in a significantly higher percentage of forwardly progressive spermatozoa at the 4th hour; e) the addition of propranolol resulted in a dramatic decrease of motility.

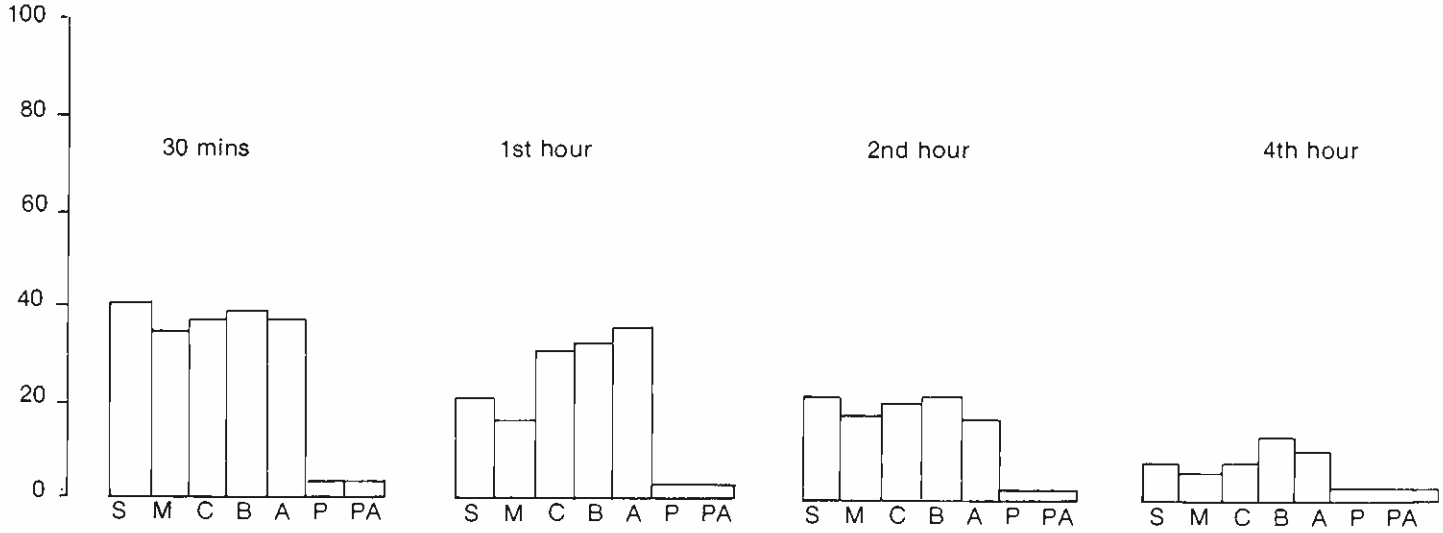
These results suggest that pentoxifylline increases the duration of activity of ejaculated human spermatozoa. These effects could be related to an increase in the intracellular content of cAMP owing to the phosphodiesterase inhibitory effect of the drug.

2. Dose response effect of pentoxifylline on the motility of normal human spermatozoa.

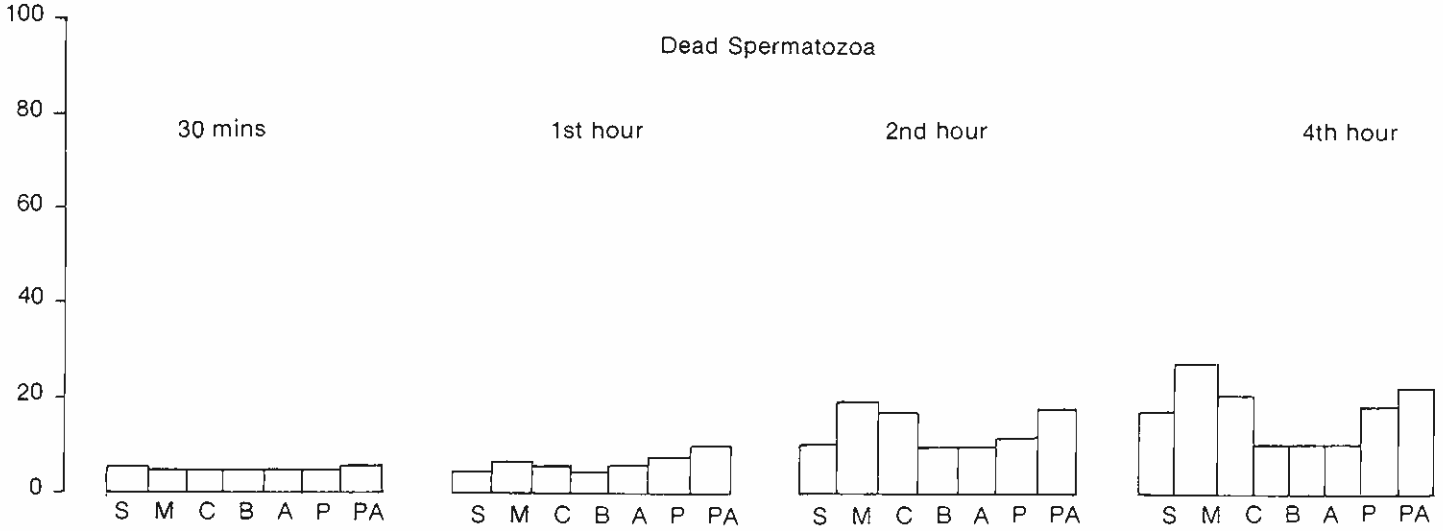
Since the study previously commented suggested that pentoxifylline could improve the motility and particularly the length of activity of ejaculated human spermatozoa, this study was performed to try to confirm those findings and to try to see if the effect is dose dependent. Semen samples of six normal volunteers were used. The technical procedure was in general terms similar to the one described in the previous study with the only exception that the fluid for dilution was in this case Tyrode fluid instead of Mann-fructose fluid. Pentoxifylline was added to obtain respectively a final concentration of

Fig. 2

Forwardly Progressive Spermatozoa



Dead Spermatozoa



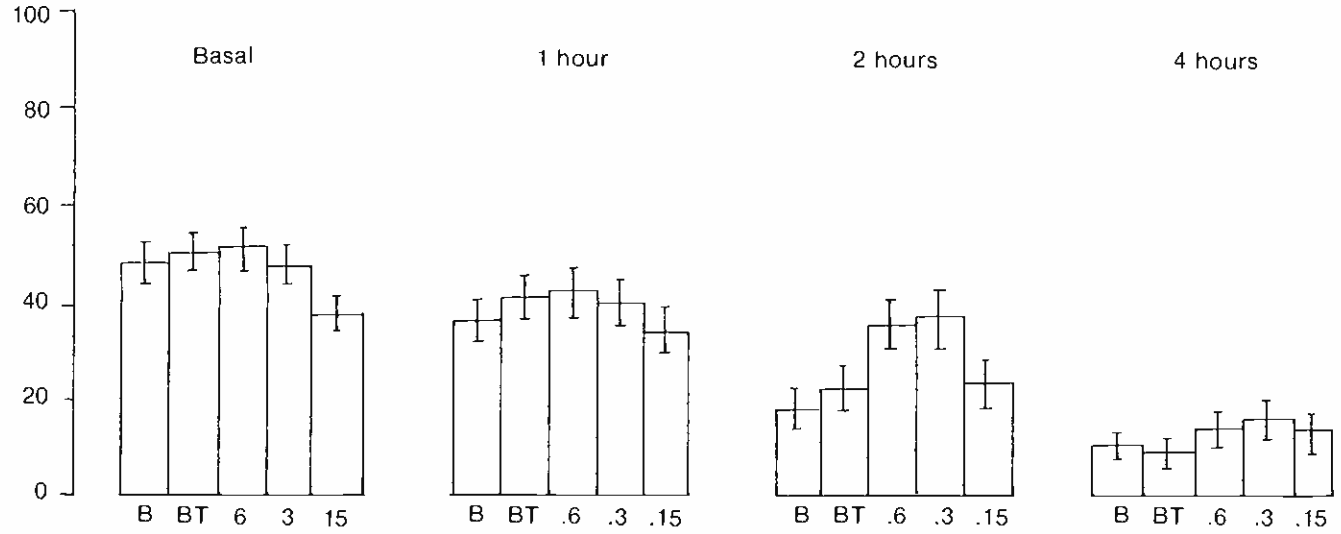
S: Semen alone: M: Semen + Mann — Fructose Fluid: C: Semen + Mann — Fructose — Caffeine

B: Semen + Mann — Fructose + BL 191; Semen + Mann — Fructose + cAMP.

P: Semen + Mann — Fructose + propranolol; PA: Semen + Mann — Fructose + propranolol + cAMP.

0.15, 0.30, 0.60 mM. As it can be seen in Figure 3, the motility decreased in the control semen up to 4th hour. The addition of Tyrode fluid did not modify significantly the findings in comparison to control group. The addition of pentoxifylline particularly at the concentration of 0.3 and 0.6 mM determined higher percentages of forwardly progressive spermatozoa as compared to control groups, particularly at the 2nd and 4th hour.

Fig. 3: Normals-Forwardly Progressive Spermatozoa



B : Control semen

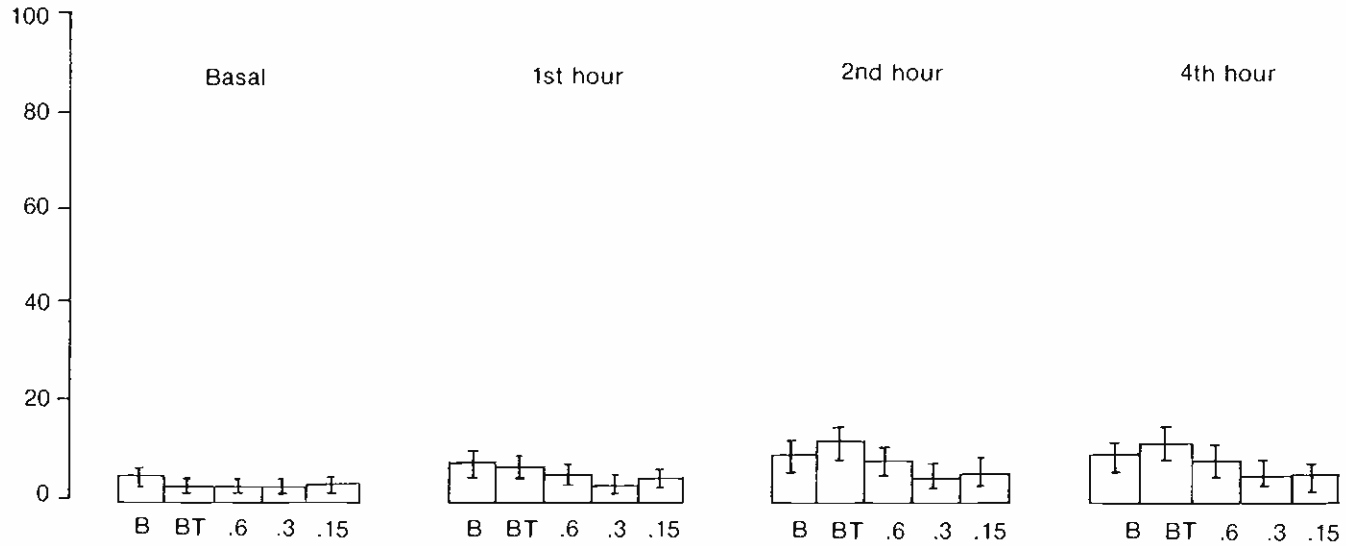
BT : Semen — Tyrode fluid

.6, .3, .15 : Concentration(mM) of pentoxifylline

Figure 4 represents the percentage of dead spermatozoa. The control group and the group added with Tyrode fluid alone showed significantly higher percentages of dead spermatozoa at the 2nd and 4th hour than the groups treated with pentoxifylline

These findings confirm the possibility of using pentoxifylline to induce an extension of duration of activity of human ejaculated spermatozoa opening the possibility of a sperm vitalizing therapy.

Fig. 4: Normals-Dead Spermatozoa



B : Control semen

BT : Semen — Tyrode fluid

.6, .3, .15 : Concentration(mM) of pentoxifylline

3. Effect of pentoxifylline on spermatozoa of patients with asthenospermia.

On the basis of the previous studies, the next step in the research concerning possible uses of pentoxifylline in infertility was to assess its effects "in vitro" in semen samples of asthenospermic patients. The selection of patients included six men considered to have idiopathic normogonadotropic oligoasthenospermia on the basis of: 1) three spermograms performed with at least one month interval between them, showing less than 30% of motile spermatozoa with normal biochemical parameters in semen and no evidence of infection and/or contamination with Mycoplasma. 2) no evidence of endocrinological disorders at the hypothalamic pituitary gonadal axis nor extragonadal. 3) good general clinical condition of the patient. 4) absence of varicocele or any other urologic disease. 5) homologous immunological test showing no aggressivity of seminal plasma and/or blood plasma of the patient to his own spermatozoa. Eventual genetic disorders were not excluded in these patients.

The methodology used to assess the effects of pentoxifylline was the same described for the first study. Figure 5 shows the results concerning forwardly progressive spermatozoa, forwardly and slowly progressive spermatozoa and dead spermatozoa. In basal conditions (that is immediately after liquefaction) the semen added with Tyrode fluid showed higher percentages of forwardly progressive spermatozoa than semen alone. Semen added with pentoxifylline at different concentrations did not differ at this point from semen added only with Tyrode fluid. However, after 1, 2 and 4 hours added with pentoxifylline showed significantly higher percentages of forwardly progressive spermatozoa and forwardly and slowly progressive spermatozoa than control semen and semen added with Tyrode fluid. The semen added with Tyrode fluid on the other hand, showed higher percentages of dead spermatozoa particularly at the 2nd and 4th hour. The results of this study suggest that pentoxifylline increases the length of activity of human ejaculated spermatozoa even in cases of severe asthenospermia as is the case in these patients. The results presented in this study are obviously the consequence of a frank improvement of motility in three out of the six samples. The other three showed less pronounced modifications. The result obtained with pentoxifylline as a tool for an *in vitro* sperm vitalizing therapy should be carefully analyzed because it could be possible that its usefulness covers only cases of intrinsic motility deficiency while in case of genetic disorders there might be no effect. Nevertheless, pentoxifylline seems at the moment a drug of choice for a sperm vitalizing therapy, since it has proven more effective than other methylxantines.

4. Use of pentoxifylline in homologous artificial insemination with semen samples of patients with asthenospermia.

On the basis of the previous studies, a trial was initiated to observe if a sperm vitalizing therapy with pentoxifylline could improve the results of homologous artificial insemination in infertile couples. It is well known that the results of this kind of insemination without any addition are disappointing.

The use in recent times of a cup device seemed to increase the results of homologous artificial insemination.

Up to now, the procedure was applied in five couples in which the women were eumenorrheic with ovulation confirmed repeatedly by endometric biopsy, urinary pregnandiol determination and body temperature curve. Vaginal conditions were normal according to bacteriological studies. No immunological aggressivity of the cervical mucus of the women was observed. The men of the couples were considered to have idiopathic normogonadotropic oligoasthenospermia according to the criteria previously commented. Homologous artificial inseminations were performed the day in which the cervical mucus score of the woman reached 9 or more and in the three successive days. Artificial insemination was performed by means of a cup. Each couple was submitted to four series according to the following scheme:

Series 1 : artificial insemination according to classical procedures. Series 2: artificial insemination without any addition performed with a cup. Series 3: artificial insemination performed with a cup and with the semen diluted with Tyrode fluid one part to five parts of semen. Series 4: artificial insemination with a cup and the addition of pentoxifylline at a final concentration of 0.3mM. Evaluation of the results of the artificial insemination was performed by means of a fractioned post-coital test (FPT) observing the number and characteristics of spermatozoa in the cervical duct. Table 1 shows the results obtained in the five couples. As it can be seen the use of the cup increased *per se* the results of the homologous artificial insemination. The addition of pentoxifylline determined a further increase of the result. Two of the patients showed motile spermatozoa. Table 2 shows overall result obtained in the five couples. An improvement of the fractioned post-coital test was observed in three cases.

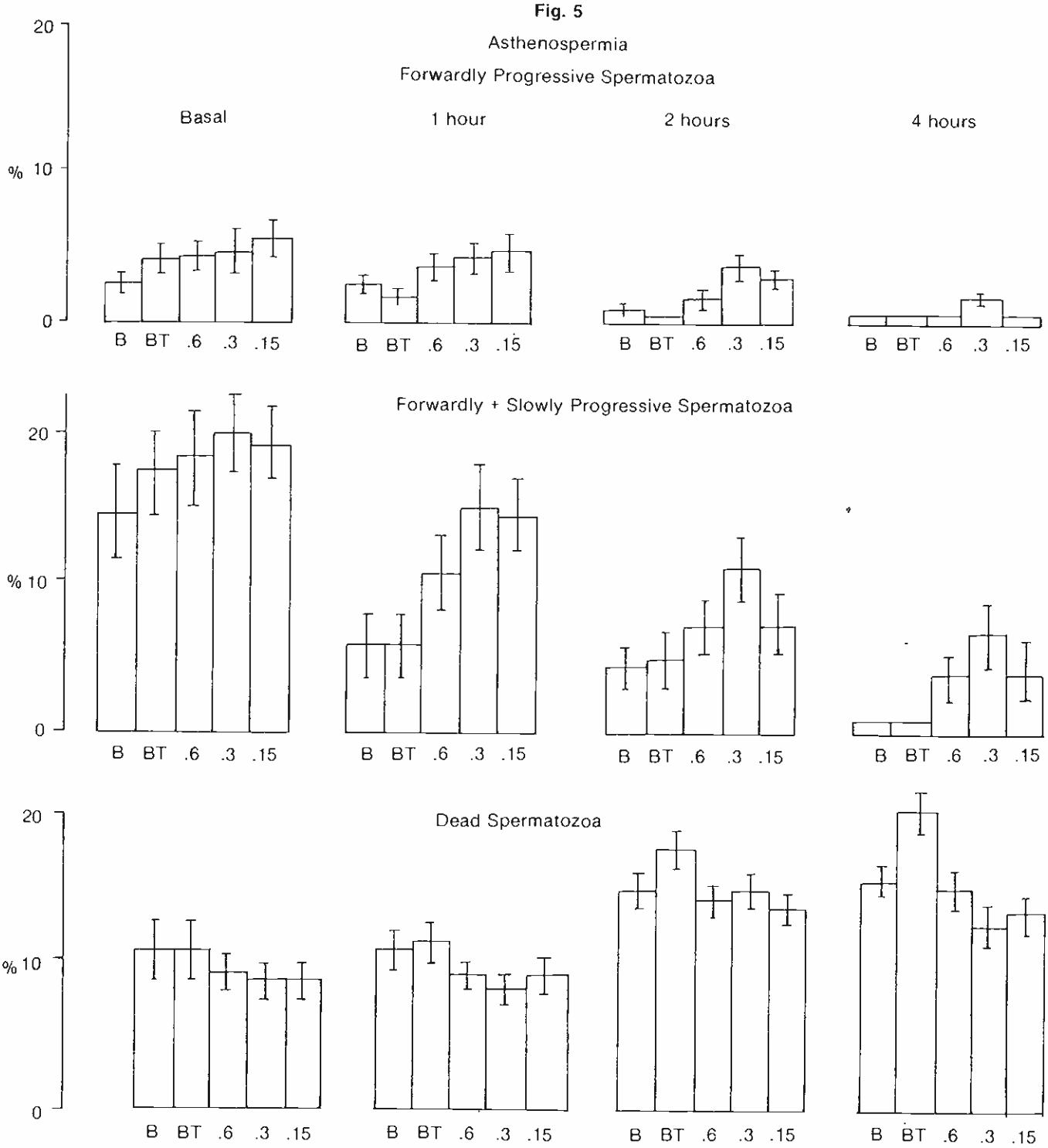
These results must be confirmed by the treatment of a greater number of cases. Anyhow, they could be considered as promising taking into account the low rate of good results generally obtained with homologous artificial insemination.

5. Treatment with pentoxifylline by oral administration in patients with asthenospermia.

This study was performed in 10 men (aged 23 to 48 years) considered to have idiopathic asthenospermia according to criteria previously commented. Each subject was submitted to a treatment with pentoxifylline *per os* at a dose of 1200 mg/day (3 times 400 mg). Assessment of the results was performed by serial spermograms performed during, at the end and up to three months after treatment. Figure 6 shows average results obtained in the 10 patients. A certain increase of forwardly progressive spermatozoa and of slowly progressive spermatozoa was observed. Table 3 shows individual

results and the overall assessment of the results. As it can be seen, frank improvement (that is to say normalization of motility parameters at the end of treatment) was obtained in three subjects while improvement (that is to say at least a duplication of the initial figures) was obtained in four. Three patients did not show significant changes. These results suggest that a treatment with pentoxifylline could be attempted in patients considered to have idiopathic normogonadotropic oligoasthenospermia. This is of particular interest due to the fact that the treatment of this entry is often difficult and disappointing even with drugs which act by stimulating directly (i.e. HMG + HCG) or indirectly (LH-RH) the testicular function. Four of the patients treated with pentoxifylline reported spontaneously increased libido.

Fig. 5



B : Semen

BT : Semen & Tyrode

.6, .3, .15 : Concentration (mM) of pentoxifylline

Fig. 6

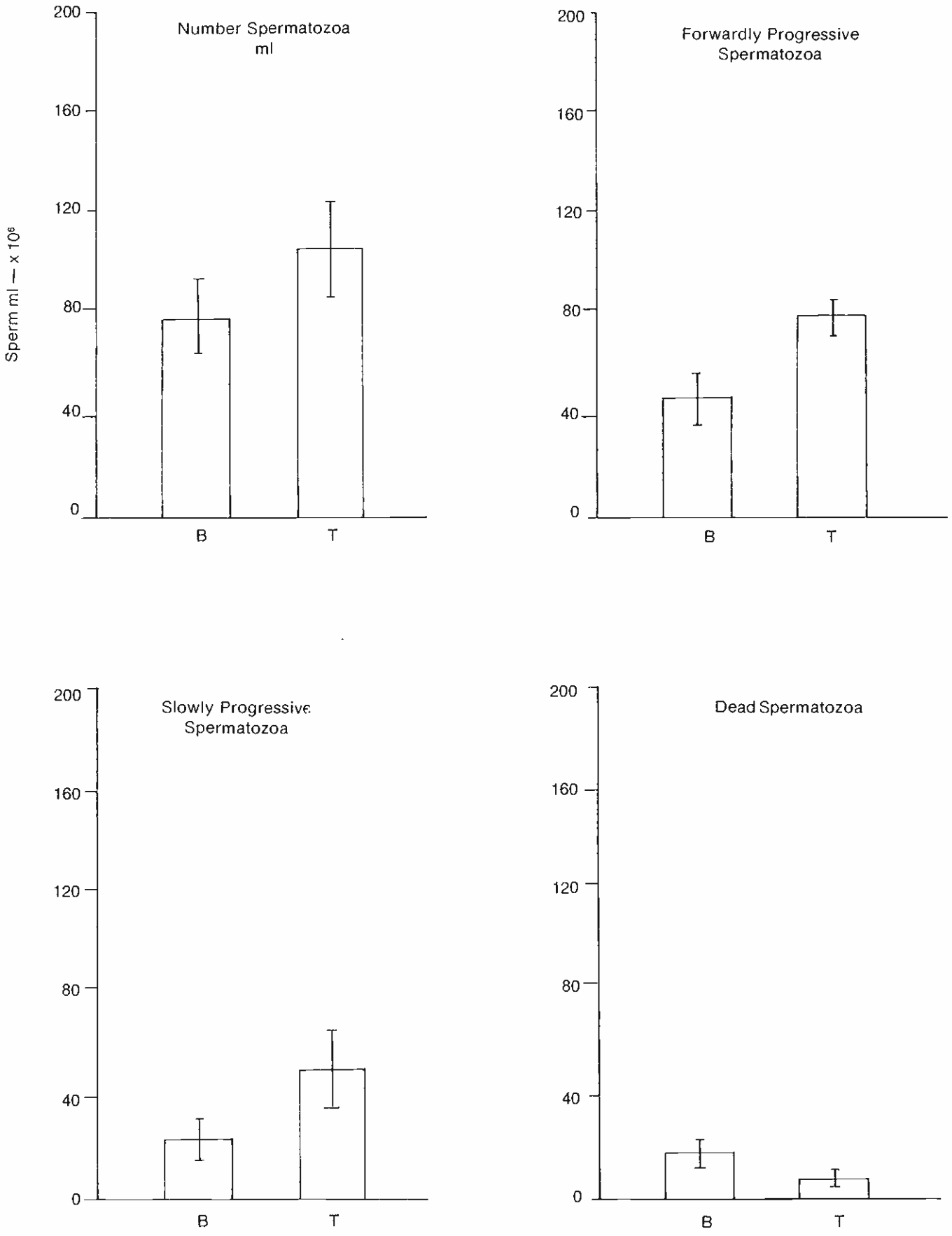

Number Spermatozoa
ml
Sperm ml — x 10⁶
200
160
120
80
40
0
B
T
Forwardly Progressive
Spermatozoa
200
160
120
80
40
0
B
T
Slowly Progressive
Spermatozoa
200
160
120
80
40
0
B
T
Dead Spermatozoa
200
160
120
80
40
0
B
T

Table 1

Effect of BL 191 on Homologous Artificial Insemination

Clinical Material: Couple with long standing sterility (>3 years) with normal women and asthenospermia men (genetic causes not excluded)

Couple No:	FPT Basal	FPT Cup alone	FPT Cup + Dilution	FPT Cup + Dilution + BL 191
1	Neg	Neg	Neg	3 non motile sp
2	Neg	2 non motile sp	2 non motile sp	3 non motile sp
3	Neg	2 non motile sp	2 non motile sp	4 motile sp
4	1 motile sp	1 motile	1 motile sp	5 motile sp
5	Neg	Neg	Neg	Neg

Table 2

Overall Results Obtained with BL 191 in HAI Performed in 5 Couples

n	FPT Improved	FPT Without changes	FPT Worse
5	3	2	0

Table 3

Patient No:	Sperm/ml		Forwardly progressive sperm		Slowly progressive sperm		Dead spermatozoa		Overall assessment of result
	Basal	BL 191	Basal	BL 191	Basal	BL 191	Basal	BL 191	
1	100	68	29	75	43	20	9	3	Frank Improvement
2	25	30	7	15	30	40	5	14	Improvement
3	120	206	0	5	0	38	26	1	Improvement
4	50	49	5	15	19	35	1	0	Improvement
5	8	31	0	11	12	40	11	10	Improvement
6	150	160	14	69	45	48	1	3	Frank Improvement
7	14	38	5	30	10	40	10	3	Frank Improvement
8	56	56	3	5	27	40	15	6	No change
9	62	108	10	16	25	39	5	15	No change
10	200	290	38	10	32	58	10	1	No change
$\bar{X}$	78.5	103.6	11.1	25.1	24.3	39.8	9.3	5.6	
S.E.M.	18.9	26.5	3.8	7.7	4.3	2.8	2.2	1.6	

6. Artificial insemination in cows using pentoxifylline.

As an ancillary program to the use of pentoxifylline in infertility, a trial was performed in artificial insemination in cows. 487 cows were submitted to one artificial insemination without any addition while 494 were submitted to the same procedure but with the addition of pentoxifylline at a final concentration of 0.3mM. The allocation of the cows to both groups was performed at random during the months of May, June, July and August (Winter in Argentina) and using the same semen. All were cows of Aberdeen Angus type of the same age. Table 4 shows the result obtained. As it can be seen, the group without pentoxifylline achieved a pregnancy rate of 60% which is really high for the first insemination. The group treated with pentoxifylline achieved a pregnancy rate of 71%. This rate is extremely high for one insemination. These results suggest that the addition of pentoxifylline could be useful for veterinary procedures allowing for increasing the pregnancy rate and to decrease the number of inseminations necessary to obtain a satisfactory pregnancy rate.

Table 4

Rate of Pregnancy Obtained During AI in Cows With or Without the Addition of BL 191 to the Fluid

	Without BL 191	With BL 191
n	487	494
No. pregnant after 1 insemination	292	350
%	60.0	71.0

GENERAL CONCLUSIONS

From the result reported it can be concluded that pentoxifylline can be a beneficial tool for the management of certain cases of human infertility as an additive for a sperm vitalizing therapy or even as a treatment for asthenospermic patients. Also it showed to be useful for artificial insemination in domestic animals. Further trials, particularly in human application, are necessary to put these remarkable findings on a wider basis.

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