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·Complementary Medicine·

Significant reduction of sperm disomy in six men: effect of traditional Chinese medicine?

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Abstract

Aim: To test the hypothesis that levels of sperm disomy fell significantly in six men treated by traditional Chinese medicine (TCM). **Methods:** Fluorescence *in situ* hybridization (FISH) was done on the sperm heads of six men before and during treatment by TCM. **Results:** There was a significant reduction in sperm disomy in all six men. This coincided with TCM treatment. **Conclusion:** This is the first study reporting a significant reduction in sperm disomy in men over a given time course. The fact that this coincided with TCM treatment is intriguing but no conclusions can be drawn from this until placebo-controlled clinical trials are implemented. *(Asian J Androl 2005 Dec; 7: 419–425)*

Keywords: traditional Chinese medicine; sperm disomy; chromosome; male infertility

1 Introduction

Sperm aneuploidy is the presence of one or more extra or missing chromosomes in the sperm head. Because of the difficulties of probe hybridization with distinguishing nullisomy (absence of a chromosome), disomy (an extra chromosome) is usually only noted [1]. The level of sperm disomy therefore can be defined as the proportion of sperm with an extra given chromosome. A number of factors have been associated with increased levels of sperm disomy including age [2], smoking [3], chemotherapy [4] and, more significantly, severely compromised semen parameters [1]. Men with abnormal levels of sperm concentration, motility and/or morphology (e.g. severe oligoasthenoteratozoospermia [OAT]), have been shown to have up to a 30-fold increase in levels of sperm disomy compared with normal controls [1]. This is by far the most significant correlate of increased sperm disomy; for example, by comparison, an increase in age from 25 to 55 correlates to only a two-fold increase in sperm disomy for the sex chromosomes [2].

Men with severely compromised semen parameters, e.g. severe OAT (and thus often a high incidence of sperm disomy), are frequently treated by intracytoplasmic sperm injection (ICSI). The subsequent potential for non-Mendelian transmission of diseases such as Klinefelter Syndrome and Down Syndrome, as well as the increased propensity for ICSI failure through injection of a disomic sperm, has raised considerable debate in both the scientific and popular press [1, 5]. To date, however, clinical intervention has been limited to screening for defects with a view to genetic counseling of individuals with abnormally high levels about 1) the risks of

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affected children and 2) the likely success of the ICSI procedure itself [5]. To our knowledge, there have been no studies reporting that these high levels in infertile men can, potentially, be reduced. In this paper, we assayed sperm disomy levels of six men at various time points. By coincidence, all were under treatment for infertility by traditional Chinese medicine (TCM). TCM originated in China over 5 000 years ago and is still used as a primary therapy in a number of Asian countries, often in parallel with Western medicine [6]. The diagnostic techniques and therapeutic philosophy in TCM differ from Western medicine in that, rather than target specific biochemical pathways, they define health in terms of balance. Simplistically, TCM is based around the doctrines of Yin-Yang that are defined as two fundamental principles that oppose and complement each other and are present in all things. Yin-Yang is a balance between the anabolic and catabolic processes, and healthy physiology and metabolism depend on the "vital energy", or Qi that flows throughout the body [6]. Thus both the body in isolation and that in relationship to the environment should be balanced, and any alteration in this balance (between Yin and Yang) results in disease. The application of TCM either in the form of acupuncture or herbal formulations has been used to treat a range of infertility phenotypes, primarily those affecting female infertility and male infertile factors, including varicocoele, seminal plasma abnormalities and semen parameters. Studies carried out using TCM have been shown to significantly improve the quality and quantity of sperm (for a review of the TCM treatment of female and male infertility see Xu *et al*. [6]). To the best of our knowledge, this is the first study performed to assess sperm aneuploidy levels as a result of, or coincident with, any medical intervention.

2 Materials and methods

Six men undergoing TCM treatment for infertility at the Zhai Clinic, Harley Street, gave informed consent for the levels of sperm chromosome abnormalities to be assayed during the time that they attended the clinic. Controls were normal and OAT donors who did not receive TCM treatment. The age of patients and controls ranged from 33 to 39 years with a mean of 35.5 years; none of the patients were smokers nor drug-users, and none were receiving chemotherapy treatment. Individuals were given a physical examination, and their semen quality was assessed according to the WHO guidelines and Kruger strict criteria for assessment of morphology. The TCM treatment that they received consisted of a cocktail of 10–20 herbs (taken as an infusion). A list of the herbs is given in Table 1, (for more information contact the Zhai clinic, 112 Harley Street, London, W1N 1AF, UK; Tel: +44-207-9083-866; E-mail: zhaiclinic@btopenworld.com). In line with TCM practice the composition of the treatments and the doses varied depending on the results of the physical examination, which included examinations of the patients' pulse, diet, appearance of the tongue, lifestyle and semen assessment. That is, patients took 5 g and 15 g of each herb, mixed together in a 1-L infusion.

Sperm disomy was assessed according to Griffin *et al*. [2, 7] for chromosomes X, Y and 21 (trisomies of which can lead to Klinefelter or Down Syndrome). All sperm samples were taken on the day of donation, resuspended three times in buffer (10 mmol/L Tris. HCl, 10 mmol/L NaCl) then spread on to a glass slide. Fluorescence *in situ* hybridization (FISH) followed standard protocols, (i.e. swelling using 10 mmol/L Dithiothreitol [DTT]), simultaneous denaturation of probe and target at 72 ºC followed by formamide stringency washes and 4,6 Diamidinophenylindole (DAPI) counterstain. Probes used were supplied by Vysis (Downers Grove, IL, USA) and labeled with spectrum orange (Chromosomes 21 and X) and spectrum green (chromosomes X and Y). Thus, chromosome 21 appeared in red, X chromosome in yellow and Y chromosome in green. The total number of 5 000 sperm was chosen in line with the majority of studies that have investigated the association between sperm disomy and compromised semen parameters [1]. The number of sperm with an extra one of these chromosomes (out of 5 000) was noted for each sample using a Leica epifluoresence microscope equipped with Photometric CCD camera and Smart Capture software (Digital Scientific, Cambridge, UK). Samples were taken at entry into the clinic then at subsequent intervals between 6 weeks and 6 months.

The results were controlled in three ways. First, disomy levels were compared to samples from each of the four control males who were of proven fertility (a total of nine samples); this was designated control group 1. Second, patient disomy levels were compared to one sample from each of the four males with severe OAT (control group 2). Finally, all disomy levels for each patient were compared with the first sample and the sample immediately preceding it to address the question of whether the disomy levels had fallen. All preparations

Table 1. Latin and common names of the herbs used in the TCM treatments

Latin name	Common name
Radix paeoniae rubrae	Red peony root
Radix cyathula officinalis	Cyathula root
Sclerotium poriae cocos	Poria
Fructus lycii chinensis	Lycium fruit
Cortex phellodendri	Phellodrendron bark
Cortex moudan radicis	Moutan
Fructus ligustri lucidi	Ligustrum seed/glossy privet fruit
Rhizoma dioscoreae	Dioscorea root
Fructus corni officinalis	Cornus fruit/dogwood fruit
Semen persicae	Persica
Semen cuscutae	Dadder seed/cuscuta seed
Rhizoma alismatis plantago-aquaticae	Alismatis rhizome/water plantain tuber
Rhizoma anemarrhenae asphodeloidis	Anemarrhena
Radix moridae officinalis	Morinda root
Rhizoma atractylodis macrocephalae	Alba atractylodes
Semen plantaginis	Plantago seed
Radix codonopsis pilosulae	Codonopsis
Fructus rubi	Rubus
Radix glycyrrhizae uralensis	Liquorice root
Flos carthami tinctorii	Safflower flower
Semen varricariae pyramidata medic	Vaccaria seed
Fructus schisandrae chinensis	Schisandra fruit
Aurantii fructus	Aurantium fruit
Acori graminei rhizoma	Acorus rhizome
Nelumbinis embryo	Lotus embryo
Rhizoma dioscoreae septemlobae	Hypoglauca yam
Rhizoma atractylodis	Attractylodes
Radix bupleuri chinensis	Bupleurum
Rhizoma cimicifugae	Bugbane rhizome
Radix astragali seuhedysari	Astragalus root
Radix rehmanniae glutinosae	Fresh rehmannia
Pericarpium citri reticulata blanco	Citrus peel
Flos lonicerae japonicae	Lonicera flower
Herba epimedii	Epimedium
Semen coicis	Coix seed/jobs tear seed

were scored "blind" by at least two independent observers (i.e. without prior knowledge of the origin of the sample); strict scoring criteria [2, 7] were applied.

3 Results

In all six patients, initial samples were significantly greater than the mean of control group 1 (normal) and comparable to the mean of control group 2 (severe OAT). Sperm disomy subsequently fell to levels not significantly different to that of the mean of control group 1. That is, levels in the final sample were all significantly lower than the initial sample when all chromosomes were taken into account. Table 2 showed the levels for each chromosome pair; Table 3 showed the levels collectively for all chromosomes assayed as well as information about the semen assessments. Patients had varying degrees of aberrant semen parameters; however, we found no asso-

Table 2. Individual disomy levels (number of disomic sperm out of 5 000 counted) for each type of disomy (and diploidy). S1, S2, *etc.* are the sample numbers, the first number in each of the patient columns are the number of sperm scored for XX, XY, YY and 21 disomy and the final column displays the diploid results.

	Control group 1					Control group 2					Patient A					Patient B				
	XX	XY	YY	21	dip	XX	XY	YY	21	dip	XX	XY	YY	21	dip	XX	XY	YY	21	dip
S1	1	8	1	2	6	0	10	4	14	25	6	29	4	59	19	3	28	6	13	48
S2	1	3	0	3	9	2	89	10	31	77	2	16	6	6	4	0	16	7	12	26
S3	0	2	0	4	5	4	19	2	22	47	4	10	1	6	20	1	14	0	7	67
S4	1	2	0	2	10	1	8	2	15	20	2	10	0	7	11	1	3	1	11	34
S5	1	4	1	4	8						3	6	0	4	5	2	6	1	3	69
S6	1	7	0	9	12						0	6	0	3	13	1	10	1	6	50
S7	0	3	0	1	22						2	5	0	9	10					
S8	0	2	0	3	11															
S9	0	6	0	1	16															
Comment (χ^2- test)											XY, YY, 21 and overall sex chromosomes significantly reduced					XY, YY, 21 and overall sex chromosomes significantly reduced				

	Patient C					Patient D					Patient E					Patient F				
	XX	XY	YY	21	dip	XX	XY	YY	21	dip	XX	XY	YY	21	dip	XX	XY	YY	21	dip
S1	9	13	1	7	54	0	8	1	13	20	2	8	2	19	16	1	8	3	12	32
S2	1	11	0	4	22	2	7	2	5	8	0	1	0	2	2	2	2	0	2	17
S3	0	7	0	5	25	2	4	0	5	12	4	4	0	6	15	0	2	2	2	29
S4	0	3	1	7	17	1	2	0	7	19										
S5	0	10	0	2	26															
S6																				
S7																				
S8																				
S9																				
Comment (χ^2- test)	XX, diploid and overall sex chromosomes significantly reduced (Also XY apart from last sample)					XY, 21 and overall sex chromosomes significantly reduced					21 significantly reduced					XY, 21 and overall sex chromosomes significantly reduced				

ciations between semen quality and improved sperm disomy.

4 Discussion

As far as we are aware, this is the first study to report a significant improvement in the levels of sperm disomy in men with compromised semen parameters. The effects of smoking [3] and chemotherapy [4] on sperm disomy have been well reported and thus the inference was that individuals who either quit smoking or complete their course of chemotherapy treatment might return to normal levels. Indeed, Robbins *et al.* [4] reported a decline back to pre-treatment levels around 100 days after the cessation of chemotherapy treatment. In this study, however, males who began with very high levels of sperm disomy showed a significant improvement. Given that the issue of the possible transmission of chromosome abnormalities via ICSI raises significant concerns [2]; any approach that was effective in reducing sperm disomy could ultimately be used prior to ICSI to improve success rates (i.e. to increase the chances of

Table 3. In each patient: the first line denotes number of sperm (out of 5 000) with an extra sex chromosome or chromosome 21. The second line denotes the *P* value used to determine whether the difference was statistically significant compared with the sample most recently taken from this patient. The third line denotes the *P* value used to determine whether the difference was statistically significant compared with the first sample taken from this patient. The fourth line gives an indication of the semen parameters of each sample. Control group 1 (nine samples from four normally fertile men) ranged from 3 to 16 abnormal cells per 5 000 scored, with a mean of 8.25. Control group 2 (four samples each from a different oligoasthenoteratozoospermia male) ranged from 26 to 132 with a mean of 58.25. P_1, level of significance from previous sample; P_2, level of significance from first sample; A, asthenozoospermia (< 20 % normal morphology); O, oligozoospermia (< 20 million sperm/mL); OAT, oligoasthenoteratozoospermia; AT, asthenoteratozoospermia; T, teratozoospermia (> 96 % abnormal forms); N, normal semen parameters; OT, Oligoteratozoospermia; ns: not significant.

Patients	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9
Patient A	98	30	21	19	13	9	16		
P_1		$P < 0.001$	ns	ns	ns	ns	$P < 0.05$		
P_2		$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$		
Semen parameters	A	O	OAT	AT	T	T	N		
Patient B	50	35	22	16	12	18			
P_1		$P < 0.05$	$P < 0.05$	ns	ns	ns			
P_2		$P < 0.05$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$			
Semen parameters	OAT	OAT	T	AT	OT	OT			
Patient C	30	16	12	11	12				
P_1		$P < 0.05$	ns	ns	ns				
P_2		$P < 0.05$	$P < 0.01$	$P < 0.001$	$P < 0.001$)				
Semen parameters	OT	OT	T	OT	T				
Patient D	22	16	11	10					
P_1		ns	ns	ns					
P_2		ns	$P < 0.05$	$P < 0.05$					
Semen parameters	N	A	T	A					
Patient E	31	3	14						
P_1		$P < 0.001$	$P < 0.001$						
P_2		$P < 0.001$	$P < 0.01$						
Semen parameters	T	OT	T						
Patient F	24	6	6						
P_1		$P < 0.001$	ns						
P_2		$P < 0.001$	$P < 0.001$						
Semen parameters	O	T	N						
Control group 1									
Semen parameters	N	N	N	N	N	N	N	N	N
Control group 2									
Semen parameters	OAT	OAT	OAT	OAT					

injecting a chromosomally normal sperm). While we do not claim that we have yet discovered such a protocol that we have at least demonstrated that high chromosome abnormalities levels can be reduced. This leaves us to speculate on the reasons why the levels fell significantly in each patient. The association with the TCM treatment and the significant reduction coincident with this treatment is intriguing. It would be unwise at this stage to suggest that the TCM treatment itself was the reason for the improvement. Nevertheless it is not unreasonable, given these results, to propose that double-blind placebo-controlled clinical trials should ensue to test this hypothesis. Of course, good, well-designed studies should require no other justification; however, it is our opinion and experience that such a trial would be unlikely to be supported either financially or by ethical committees until a degree of evidence was found that suggested that the trial might be effective and thus of patient benefit. Indeed Yuan and Lin [8] emphasized the importance of open tube studies as forerunners for double-blind, placebo-controlled clinical trials of new pharmaceutical regimes; this was an example of such a study. In order to address the contentious issue of changing prescriptions and potential variability in herb potency, we suggested that a future double-blind placebo-controlled clinical trial should mirror that of Bensoussan *et al.* [9]. That is, the TCM practitioner would examine every patient in the traditional way and make the prescription. The first group would receive the herbs as prescribed. The second group would, unbeknown to the practitioner, receive, not the herbs as prescribed, but a predetermined combination standardized for potency that would not change. The last group would (again unbeknown to the practitioner) receive a placebo and neither patient nor practitioner would be aware of which patients were in which group. There are few reports in the literature of either natural remedies or traditional treatments having an effect on fertility [6]; however, Gurfinkel *et al.* [10] reported a positive effect of acupuncture and moxa treatment on the semen parameters of 19 men in a controlled, blind study. Moreover, St. John's Wort has been shown to have a negative effect on sperm motility if the sperm are exposed to it directly [11].

One possible explanation for our observations might be that the mechanism causing increased abnormalities involves an excess of estrogens and/or reactive oxidant species to which these males are particularly susceptible. Indeed smoking, in part through causing the formation of excessive reactive oxidative species, is believed to reduce male fertility and increase levels of sperm chromosome abnormalities, perhaps through impeding DNA repair or spindle formation mechanisms in meiosis [3]. The role of estrogen in testicular function is evident from reports of sexually mature, male, estrogen receptor knockout mice that are infertile due to seminiferous tubule dysmorphogenesis and impaired spermatogenesis [12]. Male, androgen receptor knockout mice show a steady, age-related fertility decline with disrupted spermatogenesis at early spermatid stage and Leydig cell hyperplasia; low levels of dietary phytoestrogens partially prevent these disruptions [13]. Furthermore, Feng *et al.* [14] reported a positive effect of ligustrum fruit extract on the reproductive potential of diabetic rats. While we are not aware of studies correlating endocrine disruption and sperm disomy. The action of these chemicals have been associated, sometimes controversially, with an increase in testicular dysgenesis syndrome over the last 70 years [14]. Moreover, one recent study correlated the increase in aneuploidy in endocrine disrupted mice [16]. If small quantities of endocrine disruptors or reactive oxidative chemicals were contributing to increased levels of sperm disomy in susceptible men, this might provide the basis for a mechanistic explanation as to why these levels subsequently improved in our patients. The presence of antiestrogens or antioxidants in the form of Chinese herbs might be one possibility; however, in the absence of properly controlled trials, this remains to be established. Indeed Rolf *et al.* [17] reported no significant changes in semen parameters and no pregnancies in asthenozoospermic and oligoasthenozoospermic patients exposed to anti-oxidant treatment. It might be noted, however that, in a related study (Tempest *et al.* unpublished data), we have found evidence of antiestrogenic and antioxidant activity in the herbs used to treat these men. Further studies involving controlled clinical trials and careful chemical analyses of the herbs used to treat them should make some significant steps towards establishing whether TCM does indeed have a positive effect in male fertility and sperm disomy.

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