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The Ratio of Second to Fourth Digit Length in Azoospermic Males Undergoing Surgical Sperm Retrieval: Predictive Value for Sperm Retrieval and on Subsequent Fertilization and Pregnancy Rates in IVF/ICSI Cycles

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ABSTRACT: The differentiation of the urogenital system and the appendicular skeleton in vertebrates is under the control of Homeobox (*Hox*) genes. It has been shown that this common control of digit and gonad differentiation has connected the pattern of digit formation to spermatogenesis and prenatal hormone concentrations in males. We wished to establish whether digit patterns, particularly the ratio between the lengths of the second and fourth digit in males (2D : 4D), was related to spermatogenesis and, more specifically, the presence of spermatozoa in testicular biopsies from azoospermic men undergoing surgical sperm retrieval. Forty-four men were recruited, of whom 16 were diagnosed with nonobstructive azoospermia and 4 with congenital bilateral absence of the vas deferens, and 24 previously fertile men were azoospermic after previous vasectomy. Our results show that men with previous fertility or of an acquired form of azoospermia had significantly lower 2D : 4D ratios than men with nonobstructive azoospermia. In nonobstructive azoospermia, there was a significantly lower 2D : 4D ratio on the left side in men who had successful retrieval than those with unsuccessful retrieval. For these men who had a successful retrieval, none had a 2D : 4D ratio more than 1 on the left side, whereas 4 of 7 men in whom sperm was not found had a 2D : 4D ratio greater than 1. On successful sperm retrieval, subsequent fertilization and clinical pregnancy rates were unaffected by 2D : 4D ratios.

Key words: Hox genes, finger length, azoospermia, surgical sperm retrieval.

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The vertebrate *Hox* gene family is known to be essential for limb and genital development (Herault et al, 1997; Peichel et al, 1997). The *Hox* genes are part of the Homeobox genes, and there are 4 clusters of the *Hox* gene family, *Hoxa* to *Hoxd*. The 2 most posterior genes, *Hoxd* and *Hoxa*, are required for the growth and patterning of digits and the differentiation of the genital bud (Kondo et al, 1997). It has also been demonstrated that *Hox* genes are expressed in spermatozoa after meiosis, which may indicate a role for the products of *Hox* genes in some aspect of sperm structure and/or activity (Erickson, 1990). In humans, a mutation within *Hoxa* is known to result in the condition hand-foot-genital syndrome, which is characterized by anatomical defects in digits and genitalia (Mortlock and Innis 1997). Within mice, the deregulation of *Hoxd* expression may alter the relative lengths of digits and affect the growth of the genital bud (Kondo et al, 1997; Peichel et al, 1997). These observations led Manning et al (1998) to suggest that patterns of digit growth may be related to fertility.

In the human hand, the second and fourth digits have been shown to demonstrate a pattern of approximate symmetry around the central axis of the third digit. However, there is considerable variation in the ratio of the length of the second digit to fourth digit (2D : 4D). Many individuals have longer second digits than fourth (2D : 4D more than or equal to 1), and many people have longer fourth digits than the second (2D : 4D less than 1). It has been shown that the former ratio is more common in females and the latter more common in males and that this is fixed before age 2 years and is most probably established in utero (Manning et al, 1998). It is likely that the values of 2D : 4D ratios are influenced by the concentrations of sex hormones in utero because 1) mothers with a high waist : hip ratio (a correlate of high testosterone levels) have children with low 2D : 4D ratios, 2) children with congenital adrenal hyperplasia (a trait characterized by high prenatal androgen production by the adrenal glands) have low 2D : 4D ratios (Brown et al, 2001; Okten

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et al, 2002), and 3) mothers with low 2D:4D ratios have high testosterone levels in the amniotic fluid surrounding their fetuses (Manning, 2002). High testosterone and low estrogen (low 2D:4D ratio) levels are known to favor males, whereas low testosterone and high estrogen (high 2D:4D ratio) levels are known to favor the female fetuses, which suggests the presence of ''sexually antagonistic genes'' (Manning et al, 2000). Prenatal testosterone levels are thought to have a cascade effect on developmental processes (Bardin and Catterall, 1981; Maclusky and Naftolin, 1981; McEwen, 1981; Geschwind and Galaburda, 1985).

The model thought to explain the relationship between prenatal testosterone concentrations in men and their 2D:4D ratio is that *Hox* genes control the of development both the digits and the testes. With differentiation of Leydig cells within the testes at 8 weeks' gestation, the male fetus begins to produce testosterone, and secretion reaches a maximum value at 14 weeks (George et al, 1981). Testosterone affects the development of the digits, including the 2D:4D ratio and digit dermatoglyphics. High concentrations of fetal testosterone lead to a low 2D:4D ratio, which therefore indicates high prenatal testicular activity. As a result, adult testicular activity is correlated with the 2D:4D ratio.

Prenatal testosterone is thought to be an important etiological factor in a number of developmental disorders. For example, a recent study of autism has shown that children with autism have lower 2D:4D ratios than control subjects (Manning et al, 2001). Further claims have been made that the 2D:4D ratio may be a marker or even predictor for multiple diseases, including dyslexia, migraine, stammering, immune dysfunction, myocardial infarction, and breast cancer (Manning and Bundred, 2000, 2001; Manning and Leinster 2001). There is also evidence that the 2D:4D ratio is related to early brain organization for sexual orientation (Robinson and Manning, 2000), visuospatial ability (Manning and Taylor, 2001), and ability in sports that requires good visuospatial judgment, such as soccer (Manning and Taylor, 2001).

More important for the present study is the evidence that the 2D:4D ratio is related to fertility. High sperm counts and large family size have both been found to be related to low 2D:4D ratios in men (Manning et al, 1998, 2000). In women, high 2D:4D ratios have been reported to be a correlate of large family size.

In view of the correlation of high sperm counts with low 2D:4D ratios, we wished to investigate whether a relationship could be established between a low 2D:4D ratio and reproductive success within a group of men undergoing surgical sperm retrieval (SSR) for azoospermia. A successful outcome was defined as the successful extraction of sperm and its subsequent use in assisted conception cycles. Significant proportions of these men were azoospermic after previous vasectomy, with or without failed reversal, and, as such, all had proved fertility previously and acted as control subjects. The other major group studied were men with a diagnosis of germ cell failure or nonobstructive azoospermia.

During the study, a small but important subgroup was also noted that consisted of males with congenital bilateral absence of the vas deferens (CBAVD), a condition related to the genital form of cystic fibrosis. We wished to establish whether differences existed between the 2D:4D ratios of these 2 main groups of patients and to observe any differences within the CBAVD group. We also wished to establish whether any intergroup variations within the men with nonobstructive azoospermia offered a predictive value for successful SSR. The retrieval of spermatozoa from the control group, the obstructive cases, was always successful. Finally, the effect of the 2D:4D ratio was studied in relation to the fertilization and reproductive capacity of the recovered spermatozoa when they were used subsequently for assisted conception using in vitro fertilization (IVF) with intracytoplasmic sperm injection (ICSI).

Materials and Methods

Patient Population

Forty-five consecutive men undergoing elective SSR with subsequent cryopreservation of recovered spermatozoa underwent measurement of their digits on the day of SSR. One patient was excluded from the study because of a previous partial amputation of his left index finger at the distal interphalangeal joint. All patients were diagnosed as azoospermic after 2 separate semen analyses that had showed the absence of any spermatozoa after careful analysis, including centrifugation. Patients were azoospermic because of a history of previous vasectomy (n = 24), with or without failed reversal of vasectomy, CBAVD (n = 4), or nonobstructive azoospermia or germ cell failure (n = 16). Nonobstructive azoospermia was diagnosed by previous histology, low testicular volume (less than 10 mL), and elevated levels of serum follicle-stimulating hormone (FSH; more than 15 IU/L). All serum levels of FSH, luteinizing hormone (LH), and testosterone were done at the same time of day, to avoid any effect of diurnal variation. Of the patients with nonobstructive azoospermia, 8 had idiopathic or congenital azoospermia, 2 had a diagnosis of maturation arrest, and 6 had acquired azoospermia secondary to infection, chemotherapy, or orchidopexy. In all cases (28/28) of obstructive azoospermia, either secondary to previous vasectomy or from CBAVD, spermatozoa were successfully retrieved. Of those men with nonobstructive azoospermia, 9 (56%) of 16 had successful retrieval. In total, 30 men were diagnosed with an acquired azoospermia (24 after vasectomy, 1 after chemotherapy, 4 after orchidopexy, and 1 after immune orchitis). The remaining 14 men were diagnosed with a congenital or presumed genetic cause of azoospermia (4 with CBAVD, 2 with maturation arrest, and 5 idiopathic). Of the chromosomal

abnormalities, there was 1 man with Klinefelter syndrome 47XXY, 1 man with 46XX+SRY, and 1 man with a Y deletion.

Forty cycles of IVF/ICSI were done for 31 couples using cryopreserved epididymal or testicular spermatozoa. The resulting fertilization and clinical pregnancy rates were noted, as were the number of oocytes injected per patient and the number of grade 1 embryos.

Digit Measurement

Digit length was measured on the ventral surface of the hand from the basal crease of the digit to the tip, using vernier callipers measuring to 0.05 mm. Digit lengths are easily and reliably measured (Manning, 1995), and they are readily accessible. To minimize measurement error, each measurement was done twice and the mean value calculated for each finger of the right and left hand.

Sperm Retrieval Methods

If a palpable epididymis was felt, then Percutaneous Epididymal Sperm Aspiration (PESA) was done using the standard method described by Craft et al (1995). After the epididymis had been identified, it was secured between the thumb and index finger, and the remaining fingers and palm were used to cup and stabilize the testicle. Sperm were aspirated blindly from the epididymis via the percutaneous puncture of the epididymis with a 19-gauge needle with suction and obtained using a 10-mL syringe. The procedure was done under general anesthesia, and the epididymal fluid aspirated at the PESA was immediately examined under ×400 bright-field microscopy for the presence of motile spermatozoa. If no motile spermatozoa were seen, the patient proceeded directly to Testicular Sperm Extraction (TESE) under the same anesthetic. Testicular extraction was also done without a prior attempt at PESA if no palpable epididymis was identified or if the patient had had a previous unsuccessful PESA.

TESE was done as an open biopsy in which a small incision was made in the scrotal skin and the layers of tissue opened through the spermatic fascia and the tunica vaginalis to the tunica albuginea. After opening the tunica albuginea, seminiferous tubules were removed in 3 separate biopsies. TESE was confined to the largest testicle and was not done as a bilateral procedure. To extract the usable spermatozoa from the tubules, the tissue was minced and the tubes ''milked'' with sterile needles (Tucker et al, 1995) to achieve the mechanical isolation of spermatozoa for ICSI.

Oocyte stimulation and retrieval, ICSI, and embryo transfer—Female patients underwent a standard long-protocol IVF regimen within 2 months of successful SSR. Pituitary desensitization was with a gonadotrophin-releasing hormone agonist (Buserelin, Hoechst, Germany) and ovarian stimulation with human menopausal gonadotrophin hMG (Menogon; Ferring, Kiel, Germany). Retrieved oocytes were processed in the laboratory as described elsewhere (Hillier et al, 1984) and cultured in P1 medium supplemented with 10% v/v SSS (IVF Science, Goteborg, Sweden) under an atmosphere of 5% CO_2 in air at 37°C. After approximately 4 hours of incubation, the oocytes were mechanically denuded of their cumulus and coronal cells aftter a brief exposure to hyaluronidase (Sigma Chemical Company, St Louis, Mo).

Spermatozoal preparation—Epididymal spermatozoa were washed by centrifugation at 600 × *g* in P1 medium for 10 minutes, followed by resuspension in 1 mL of fresh medium. Testicular spermatozoa extracted from the seminiferous tubules were washed by centrifugation at 600 × *g* in P1 medium for 10 minutes, followed by resuspension in 3 mL of fresh medium and overnight culture. Samples were then cryopreserved for future use in a 1:0.7 dilution of sperm freezing media (SpermFreeze; FertiPro, Beenem, Belgium) in heat-sealed 0.5-mL straws using the slow-freeze method (Mahedevan and Trounson, 1993) in a planar Biomed controlled-rate freezer (TS Scientific, Perkasie, Penn). Prior to use, a straw of cryopreserved spermatozoa was thawed rapidly at 37°C for 10 minutes. Cryoprotectant was removed with the dropwise addition of P1 medium, followed by centrifugation at 600 × *g* for 10 minutes and resuspension in 0.5 ml of fresh medium.

Only motile spermatozoa with normal morphology were used for ICSI. They were transferred to PVP (ICSI-100; IVF Science, Goteborg, Sweden) for immobilization prior to injection. Injected oocytes were cultured in P1 medium and examined for the presence of pronuclei approximately 18 hours after ICSI.

Immediately prior to transfer of the embryos, they were all assessed and graded according to standard criteria (Scott et al, 1991). A maximum of 3 embryos were transferred into the uterus approximately 48 hours after ICSI. Successful implantation was determined by positive urinary ß-HCG and a clinical pregnancy test by the presence of a fetal heart beat on vaginal scan 4 to 6 weeks after embryo transfer.

Statistical Analysis

We used model II single-factor analysis of variance (ANOVA) tests to calculate the intraclass correlation coefficients or repeatability (r_1) of our measurements (r_1 = groups MS − error MS/groups MS + error MS, where MS is the mean square; Zar, 1985). Repeated-measures ANOVA tests were used to calculate the ratio *(F)* between groups MS (ie, differences between individuals) and error MS (ie, measurement error). Repeatability was high, and the variance of our between-subject measures was significantly greater than the variance of our repeated measures (left hand $r_1 = 0.98$, $F = 104.38$, $P = .0001$; right hand $r_1 = 0.99$, $F = 135.80$, $P = .0001$). We concluded that our measurements of 2D:4D ratios showed real differences between subjects.

Statistical analysis was then done using parametric tests that included factorial and repeated-measures ANOVA for multiple analysis, with post hoc analysis after Bonferroni transformation and independent *t* tests to compare digit ratios and fertilization rates. Pregnancy rates were analyzed using Fisher's exact and χ^2 tests. Bivariate analysis was used to examine relationships between fertilization rates and 2D:4D ratios. $P < .05$ was taken to indicate statistical significance.

Results

Given the small numbers (4 subjects) within the subgroup of CBAVD, the major comparisons of the effect of etiology on 2D:4D ratio was compared between men with a history of previous vasectomy (ie, previous fertility) and

Table 1. *Mean ± SD of the relationship of the 2D:4D ratio in azoospermic men to diagnosis*

	Vasectomy	CBAVD	Germ Cell Failure
Number of patients	24	4	16
2D:4D left	0.97* ± 0.035	0.98 ± 0.002	0.98 ± 0.030
2D:4D right	0.97* ± 0.013	0.98 ± 0.014	0.98 ± 0.04

* $P = .16$.

those with nonobstructive azoospermia. The statistical analysis confirmed that men with CBAVD appeared to be a distinct group, as demonstrated by the high repeatability of the measurement and the small standard deviation between subjects. Men with CBAVD were included in the analysis within the larger group of men with congenital azoospermia. A history of previous vasectomy was shown to be associated with a significantly lower 2D:4D value bilaterally than nonobstructive azoospermia (0.97 vs 0.98, respectively; $P = .016$). When data from men with CBAVD were studied by ANOVA for comparison with the other etiologies, they again appeared to be a distinct subgroup (Table 1). When consideration of whether the azoospermia was congenital (cases of CBAVD, idiopathic nonobstructive azoospermia, and maturation arrest) or acquired (vasectomy, chemotherapy, or orchidopexy), there was again a significant reduction in mean values of 2D:4D ratios in acquired azoospermia compared with congenital causes (0.97 vs 0.99, respectively; $P = .015$) (Table 2).

When comparisons were made between patients from whom sperm was successfully retrieved, there was again a significant reduction in mean 2D:4D values in the left hand compared with patients who had unsuccessful retrieval (0.97 vs 1.01; $P = .001$) (Table 3). The reduction in the 2D:4D value for the right hand was not significant (0.97 vs 1.00; $P = .053$). Of far more significance was the reduction in mean 2D:4D values in the left hand in patients with germ cell failure who underwent successful SSR than those whose retrieval was unsuccessful (0.97 vs 1.01; $P = .001$). This trend was seen in the ratios of the right hand but did not reach significance. Of more prognostic value was the fact that no patients with nonobstructive azoospermia who had a successful retrieval had a 2D:4D ratio greater than 1 in the left hand, whereas more than half the patients in the unsuccessful retrieval group had a ratio greater than 1 in the left hand (0/9 vs 4/7; $P = .019$) (Table 4). Again, these results were confined to the left hand. Values for FSH, LH, testosterone, and testicular volume did not differ significantly between patients with nonobstructive azoospermia who had successful retrieval and those who had no spermatozoa recovered (Table 4).

When the results of IVF/ICSI were analyzed with respect to 2D:4D ratios in both hands, no significant differences were found with regard to fertilization or clinical pregnancy rates (Table 5). Further attempts were made to establish whether any correlation could be made to outcomes of IVF/ICSI with any dependent variable within the men, and no significant correlation to fertilization or clinical pregnancy rates could be found with 2D:4D ratios of either hand or with serum concentrations of testosterone, FSH, and LH, or with testicular volume.

Discussion

We found similar trends in right and left 2D:4D ratios, their relationships with diagnosis and nature of azoospermia, and success in sperm retrieval. However, the left 2D:4D ratio showed the strongest associations and was significantly predictive of sperm retrieval, whereas the right 2D:4D ratio was not. It may be that both left and right ratios are correlates of sperm retrieval and that the differences we have observed were due to sampling effects. Further work is needed to determine whether the left 2D:4D ratio is indeed a more reliable predictor of successful sperm retrieval than the right 2D:4D ratio.

With the successful retrieval of spermatozoa from the epididymis or testis in around 100% of cases of obstructive azoospermia (Tournaye et al, 1997), the major challenge to clinicians and scientists is the management of

Table 2. *Mean ± SD of the relationship of the 2D:4D ratio to the nature of azoospermia*

	Congenital Azoospermia	Acquired Azoospermia
Number of patients	14	30
2D:4D left	0.99 ± 0.028	0.97* ± 0.032
2D:4D right	0.98 ± 0.030	0.97† ± 0.035

* $P = .015$.
† Not significant ($P = .148$)

Table 3. *Mean ± SD of the 2D:4D ratio with regard to sperm retrieval*

	Sperm Retrieved	Sperm Not Retrieved
Number of patients		
2D:4D left	0.97* ± 0.030	1.01 ± 0.030
2D:4D right	0.97† ± 0.030	1.00 ± 0.030

* $P = .001$.
† Not significant ($P = .053$).

Table 4. *Predictive value of the 2D : 4D ratio in nonobstructive azoospermia*

	Sperm Retrieved	Sperm Not Retrieved	*P**
Number of patients	9	7	
Mean ± SD FSH, IU/L	17.7 ± 11.4	27.5 ± 20.0	NS
Mean ± SD test volume, cc	9.8 ± 2.8	9.6 ± 2.7	NS
Mean ± SD D2 : D4 right (R)	0.97 ± 0.029	1.00 ± 0.033	NS
Mean ± SD D2 : D4 left (L)	0.97 ± 0.015	1.01 ± 0.026	.001
D2 : D4 (R) > 1	3/9	4/7	NS
D2 : D4 (L) > 1	0/9	4/7	.019

* NS indicates not significant.

nonobstructive azoospermia. Two areas of major clinical and research effort are directed toward increasing the rates of retrieval of spermatozoa from the testis and identifying or predicting patients who will have a successful retrieval.

In SSR for nonobstructive azoospermia, many modifications to the technique have been made to improve recovery rates, such as microscopic dissection, multiple biopsies, or enzymatic digestion of tissue. Recovery rates remain around 50% in most centers (Kahraman et al, 1996; Salzbrunn et al, 1996; Crabbe et al, 1998; Hauser et al, 1998; Rosenlund et al, 2001). This is consistent with the results obtained in the present study. In a small study of only 20 patients with nonobstructive azoospermia, 19 had sufficient sperm retrieved for ICSI after the mapping of the testes by multiple fine-needle aspiration prior to retrieval, which suggests that sperm may be present in most men with nonobstructive azoospermia (Turek et al, 1999). At present, there are no firm noninvasive prognostic factors to predict the patients who may have recoverable sperm. Serum FSH levels, testicular size, semen biochemistry, and previous histology reports have all been used with very low degrees of success in terms of positive predictive factors for sperm retrieval (Ezeh et al, 1998; Schulze et al, 1999). Our results further confirm the poor predictive value of these parameters. Indeed, only the histology reports of tissue taken at the time of collection have been shown to have any significant predictive value (Jezek et al, 1998; Seo and Ko, 2001).

As yet, no single variable or combination of variables has been accurate enough for use as a predictive test prior to SSR. Recent data with regard to inhibin-B concentrations in 2 small studies have shown this to be a better predictor of success than FSH, but it is not yet accurate enough to use as a predictive test (Ballesca et al, 2000; Brugo-Olmedo et al, 2001).

The results reported herein have clearly demonstrated a potential link between the nature or etiology of azoospermia and 2D : 4D ratio in men undergoing SSR. Men with proven previous fertility and who were azoospermic after vasectomy had significantly lower 2D : 4D ratios in both hands than men with CBAVD or nonobstructive azoospermia. This trend was again seen when comparing men who were deemed to have acquired azoospermia with those who had what appeared to be a congenital or possibly genetic form of azoospermia. Of perhaps greatest significance for the clinician is in the consideration of 2D : 4D ratios in those men with nonobstructive azoospermia, in whom we have seen (unlike obstructive etiologies) that the level of successful SSR is not 100%.

Our results demonstrate that, in individuals in whom sperm retrieval is successful, the 2D : 4D ratio of the left hand is significantly lower than it is in those from whom spermatozoa were not retrieved. Indeed, in the successful retrieval group, no man had a 2D : 4D ratio greater than 1, whereas, in the unsuccessful group, 4 of 7 patients displayed this ''female'' pattern. Unfortunately, because 6 patients with obstructive azoospermia also had a 2D : 4D ratio greater than 1 in the left hand, the finding of a 2D : 4D ratio greater than 1 in the left hand cannot be used as a guaranteed exclusion criteria for successful sperm retrieval, although its presence severely reduces the likelihood of finding spermatozoa. These results differ from the results of previous studies that found a corre-

Table 5. *Effect of 2D : 4D (left) ratio on the IVF/ICSI outcome**

	2D : 4D < 1.0	2D : 4D > 1.0
Number of cycles of ICSI	33	7
Mean ± SD number of oocytes injected	9.7 ± 4.15	8.6 ± 4.4
Mean ± SD fertilization rate	70.3 ± 20.8	71.0 ± 30.1
Mean ± SD number of grade 1 embryos	4.2 ± 5.1	5.1 ± 3.8
Mean ± SD number of embryos transferred	2.06 ± 0.56	1.86 ± 0.38
Clinical pregnancy rate, % (n/N)	15 (5/33)	43 (3/7)

* *P* was not significant for all data.

lation of 2D:4D ratios with testosterone concentrations and sperm concentration predominantly in the right hand (Manning et al, 1998). Other studies have also found evidence of laterality favoring the right side as a prognostic factor in dermatoglyphic asymmetry with testosterone concentrations (Jamison et al, 1993). It has been suggested that testosterone may slow the growth rate of the left side of the brain while enhancing growth of the right side, which suggests that testosterone effects could promote this laterality (Geschwind and Galuburda 1985). From the results from the present study, it appears that the 2D:4D ratios in the right hand show a trend to predicting retrieval that may be confirmed in larger studies, but it is the left hand that shows the clearest predictor of successful retrieval in nonobstructive azoospermia.

It is clear from our results that these simple measurements may be a useful tool in the initial work up of patients with nonobstructive azoospermia undergoing SSR. It may be also useful in identifying patients in whom a more thorough testicular sampling, possibly using fine-needle mapping (Tournaye et al, 1998) may be useful. Further larger studies should also be done in relation to the chromosomal screening of men undergoing SSR. All of the patients with nonobstructive azoospermia in our study underwent blood karyotype examination. The men did not, however, undergo microdeletion screening of their Y chromosomes. Of these karyotype results, only 1 patient had an abnormal finding (46XX, SRY). This karyotype was associated with the highest 2D:4D ratio, 1.06 (a female-type pattern). It would therefore be interesting to note whether the 2D:4D ratios are altered in men with known chromosomal deletions either macro- or microdeletions).

Our results also show that, in IVF/ICSI cycles, the 2D:4D ratio in either hand does not correlate with outcome in terms of fertilization or clinical pregnancy rates and, as such, cannot be used as a predictor for outcome, as has been shown with the 2D:4D ratio in the right hand in natural conception (Manning et al, 2000). It may therefore be possible in future to combine a number of tests such—as previous histological findings, if available, microdeletion screening, serum inhibin B levels and 2D:4D ratios—to compute or predict the likelihood of successful spermatozoa retrieval in men with nonobstructive azoospermia. Other variables that need to be considered in predicting outcome include retrieval methods, the volume or number of biopsy samples taken, and whether these samples were unilateral or bilateral. With multiple predictive tests, more accurate prognostic predictions may be possible to counsel patients prior to any attempt at assisted conception.

It is clear from this initial study that further work on larger numbers of patients needs to be performed on 2D:4D ratios and azoospermia (especially among men with nonobstructive azoospermia and CBAVD), to further establish the predictive ability of both left- and right-hand 2D:4D ratios for successful sperm retrieval alone and in combination with other physical, biochemical, and chromosomal markers of testicular function.

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