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DOES EPIDIDYMAL LENGTH IN MEN WITH CONGENITAL BILATERAL ABSENCE OF THE VAS DEFERENS HAVE A CORRELATION WITH THE FERTILIZATION RATE OF EPIDIDYMAL SPERM RETRIEVED BY MICROPUNCTURE TECHNIQUE?

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ABSTRACT

To investigate whether the variable length of the epididymis in men with congenital bilateral absence of the vas deferens (CBAVD) might have a correlation with fertilization and pregnancy rates in in vitro fertilization (IVF) treatment, we conducted a retrospective study involving a total of 60 CBAVD patients. All patients in this study had epididymal micropuncture in conjunction with perivascular nerve stimulation as part of the IVF program at the Nagoya University Hospital Reproduction Center. The patients were classified into 3 groups: group I consisted of patients having only a proximal portion of the caput epididymidis with a length of between 0.5 and 1.9 cm, group II consisted of patients with the caput and a portion of the corpus epididymidis with a length of between 2.0 and 4.0 cm, and group III consisted of patients with the caput, corpus and cauda of the epididymis measuring more than 4.0 cm. There were no differences in the sperm count, progression and normal morphology among the 3 groups; however, the motility was progressively higher in patients with a longer epididymis (12% in group 1, 18% in group II and 31% in group III). It was evident that group III showed the highest fertilization and pregnancy rate per patient (23% and 28%, respectively) among the 3 groups. This study shows that epididymal sperm from CBAVD patients with a longer epididymis have a higher IVF rate than CBAVD patients with a shorter epididymis.

Key Words: Epididymis, Length, Congenital bilateral absence of the vas deferens, IVF, Micropuncture

INTRODUCTION

Azoospermia accounts for approximately 10% of male infertility cases[1)] and connotes absolute sterility of the male partner unless treated. Unfortunately, most men with azoospermia have an untreatable testicular defect in spermatogenesis. Those who have obstructive azoospermia and intact spermatogenesis, though, are potentially treatable.

One of the more common causes of obstructive azoospermia is CBAVD,[1)] but it accounts for only 1% of the men attending infertility clinics.[2)] The estimated prevalence of bilateral vasal agenesis in the general male population is 0.05%.

Knowledge of the normal embryologic development is helpful in understanding the association of vasal agenesis with epididymal abnormalities. The testis, epididymis, and vas deferens develop during the first and second trimesters. As with the kidney, formation of a complete

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male genital tract requires the simultaneous development and joining of two separate systems.

First, the mesonephric duct joins the mesonephros early in fetal life to form a functional kidney. At approximately 8 weeks the gonadal epithelium forms both the testis cords in the cortex and rete testis in the medulla of the primitive gonad. As the mesonephros atrophies, the mesonephric tubules join the cords of the rete testis to form the efferent ductules. The efferent ductules perforate the tunica albuginea, elongate, and form the lobules of the cranial portion of the epididymis. These ductules drain into the mesonephric duct, which undergoes regional specialization to form the main genital duct. The most cranial portion becomes convoluted and is called the duct of the epididymis. The caudal portion of the mesonephric duct becomes the vas deferens. The terminal portion forms the vasal ampulla and ejaculatory duct. Thus, the epididymis and vas deferens share their mesonephric duct origin with the seminal vesicles, ureter and kidney.

Men with bilateral vasal agenesis have different degrees of epididymal development and therefore have variable epididymal length. In the majority of cases, the epididymis is formed either by the proximal portion of the caput alone or with a small proximal portion of the corpus, perhaps reflecting early developmental arrest of the mesonephric duct. Few patients show development of the caput, corpus and cauda of the epididymis.

Recently, microsurgical epididymal retrieval in conjunction with IVF has provided a new option for successful treatment of infertility in men with CBAVD.[3–6] However, success of this advanced technique does not predict fertilization and subsequent pregnancy rates. Of the many studies which have been conducted to find a parameter which could predict sperm fertilizing capability,[7–10] no report has been conclusive. Schlegel et al. suggested that the presence of a longer epididymal tubule proximal to the obstruction correlated with better sperm quality because maturation of sperm in the proximal epididymis, away from the adverse effects of dead and dying sperm in the distal epididymis, was improved.[11] Therefore, our research interest shifted to focus on the observation that men with CBAVD have different degrees of epididymal development and have different fertilization and pregnancy rates, which depend on the length of their epididymis. The aim of the present study was to investigate whether the variable length of the epididymis in men with CBAVD might have a correlation with fertilization and pregnancy rates in IVF treatment.

MATERIALS AND METHODS

A total of 60 CBAVD patients participated in this study. All patients in this study had epididymal micropuncture in conjunction with perivascular nerve stimulation as part of the IVF program at the Nagoya University Hospital Reproduction Center. The details of the surgical technique for epididymal sperm retrieval, the protocols used for ovarian hyperstimulation and the methods used for IVF have been reported in previous articles.[12,13,14] One couple underwent standard IVF, 27 couples underwent partial zona dissection (PZD) or subzonal insemination (SUZI) and 32 couples underwent intracytoplasmic sperm injection (ICSI).

For each patient the form and length of the epididymis were recorded by direct view at the time of sperm retrieval. The patients were classified into 3 groups: group I (28 patients) consisted of patients having only a proximal portion of the caput epididymidis with a length of 0.5 to 1.9 cm, group II (14 patients) consisted of patients with the caput and a portion of the corpus epididymidis with a length of 2.0 to 4.0 cm, and group III (18 patients) consisted of patients with the caput, corpus and cauda of the epididymis with a length of more than 4.0 cm. These classifications were based on Schlegel's report.[11]

Regardless of the epididymal length, all the sperm aspiration was done at the most proximal part of the epididymis i.e. in the caput or efferent ductuli, because it has been demonstrated that in this area the sperm quality is higher.[12]

All values were expressed as means ± SD. The analysis of variance followed by Tukey's test was used to compare the sperm parameters among groups. The chi square test was used for statistical analysis of fertilization and pregnancy rates among the 3 groups.

RESULTS

The total number of patients varied per group: 28 in group I, 14 in group II and 18 in group III. Table 1 shows details of the average sperm parameters (count, percentage of initial sperm motility, progression scale of motility defined by WHO criteria[15] and percentage of sperm with normal forms using strict criteria evaluation[16]) for each group. There were no differences in sperm count, progression and normal morphology among the 3 groups; however, sperm motility was progressively higher in patients with a longer epididymis (12% in group 1, 18% in group II and 31% in group III). The difference in sperm motility between patients in group I and in group III was statistically significant ($p < 0.05$).

The overall IVF outcomes for each group are shown in Table 2. In the analysis of the IVF results, only clinical pregnancies (defined by visualization of fetal heart beat on ultrasound echo) were included. The fertilization rate was defined as the number of embryos obtained per total oocytes. It is evident that patients with the longest epididymis (group III/ > 4.0 cm) had the highest fertilization and pregnancy rate per patient (23% and 28% respectively). The fertilization rate was significantly lower in group I as opposed to group III (8.3% versus 23%, $p < 0.02$). There was no statistical difference in the fertilization rate between groups I and II and groups II

Table 1 Sperm Parameters in Epididymal Fluid

Group	Sperm Count (million/mL)	Motility (%)	Progression Scale (0 to 4)	Normal Forms (%)
I (n=28)	52 ± 48	12 ± 6.2	1.2 ± 0.3	22 ± 6.5
II (n=14)	62 ± 51	18 ± 8.2	1.1 ± 0.8	26 ± 8.2
III (n=18)	49 ± 62	31 ± 7.8*	1.3 ± 0.6	24 ± 8.5

*$p < 0.05$ compared with group I.

Table 2 Overall IVF outcome*

Group	Epididymal Length (cm)	No. of Oocytes	No. of Embryos FR# (FR %)	Pregnancy
I (n=28)	0.5 to 1.9	84	7 (8.3)	1 (3.6)
II (n=14)	2.0 to 4.0	42	7 (17)	1 (7.1)
III (n=18)	> 4.0	53	12 (23)##	5 (28)##

*Values in parentheses are percentages.
Fr: fertilization rate = number of embryos obtained per total oocytes.
$p < 0.02$) compared with group I.

and III (p=0.16 and p=0.47, respectively). Similar statistical results were obtained in the pregnancy rate. This rate was significantly lower in group I as opposed to group III (3.6% versus 28%, $p < 0.02$). There was no statistical difference in the pregnancy rate between groups I and II and groups II and III (p=0.61 and p=0.14, respectively).

DISCUSSION

Our present results reveal that patients with CBAVD have different epididymal lengths and those with the longest epididymis (more than 4.0 cm) can achieve a better fertilization and pregnancy rate after epididymal sperm retrieval and subsequent IVF. The importance of the epididymis in enhancing sperm function is supported further by the higher fertilization and pregnancy rates achieved using sperm from men with a longer epididymis.

The reason why there is such a variability in the length of the epididymis is explained in a recent article by Yeung et al.[17] They describe the presence within the caput epididymidis of at least 7 types of efferent tubules connected to each other by junctions and lined by a different epithelium. Such heterogeneity of tubules and epithelia may reflect a functional role of the different anatomical regions of the epididymis.[17]

Recent evidence has indicated that more than half of all men who have CBAVD can be identified as having at least one mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene.[18] Homozygous defects in the CFTR gene are associated with the development of cystic fibrosis; however, we did not test couples CBAVD for the presence of CFTR gene mutations due to an inability to perform this experimental technique. Preimplantation genetic testing permits diagnosis of carrier embryos or homozygous embryos before embryo transfer for the most common mutation associated with cystic fibrosis, the delta-F508 mutation.[19] Hence CFTR screening is recommended for CBAVD patients to inform them of any risk of genetic abnormalities. However, in Japan, the occurrence rate of cystic fibrosis is very low compared to other races, and no cystic fibrosis was found in patients with CBAVD, or their families, in the present study. This fact indicates that cystic fibrosis may not be a critical problem in the treatment of patients with CBAVD in Japan.

Possible explanations for the better fertilization and pregnancy rates in patients with a long epididymis are as follows: [1] in a longer epididymis there is less obstructive (or less build-up back pressure) damage, and hence better epididymal functionality might be expected; [2] although the aspiration site was the same for every case, in a short epididymis the environment where sperm is collected is more heavily contaminated with macrophages, debris, and dead sperm;[8] [3] a longer epididymis (caput + corpus + cauda) has a different embryological origin, so, there might be a difference in the biochemical constitution of the epididymal fluid that could ultimately enhance the fertilizing ability of its sperm;[17] and [4] a longer epididymis can simply accommodate the arrival of more freshly produced sperm whereas the system in a short segment is completely congested and occupied by old and degenerating sperm.

In conclusion, the present study demonstrates that epididymal sperm from CBAVD patients with a longer epididymis have a better IVF rate. Therefore, the epididymal length in men with CBAVD should be routinely assessed by careful physical examination or ultrasound before epididymal sperm retrieval by micropuncture technique and IVF is performed. The information on epididymal length can be used to predict the possibility of fertilization and pregnancy when using the aforementioned advanced assisted reproductive technology.

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