FERTILITY AND STERILITY
Vol. 47, No. 1, January 1987

Printed in U.S.A.

Effects of artificial cryptorchidism on sperm morphology*

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In a diurnal artificial cryptorchidism (AC) experiment intended to provide a male contraceptive method, oligoasthenozoospermia was found as early as the fourth month, accompanied by an increase in the mean percentage of abnormally shaped spermatozoa. A detailed morphologic study concerning 19 volunteers exposed for 6 to 24 months demonstrated that the overall increase of the mean percentage of abnormally shaped spermatozoa resulted from the percentage increase of elongated, thin, and irregular heads and of bent tails. This increase is probably caused by the rise in environmental testicular temperature induced by AC. A return to baseline values was observed within 12 months after the end of the experiment.
Fertil Steril 47:000, 1987

Effects of scrotal hyperthermia on human spermatogenesis have been investigated over the last half century.[1-5] Although different heat sources and variable exposure periods were used, all previous studies have shown that testicular or scrotal heating induced a reduction of the count[1, 3-5] and motility[2] of spermatozoa. But study of spermatozoa morphologic features that are so important for fertilization, as shown by epidemiologic studies[6] and works on in vitro fertilization,[7, 8] is absent from most studies or produced discrepant results.

The first results of an experiment with testicular hyperthermia intended as a male contraceptive method were given in a previous article[9]; in this first experiment, raising the environmental testicular temperature resulted in diurnal artificial cryptorchidism (AC) that induced an oligoasthenozoospermia evidenced on average after 4 months and persisting throughout the testicular heat exposure period. This oligoasthenozoospermia was accompanied by a progressive reduction in the percentage of normal-shaped spermatozoa. Our study details the abnormalities observed in the shape of spermatozoa before, during, and after AC.

MATERIALS AND METHODS

After a 6-month baseline study, 19 healthy men volunteers (25 to 35 years old) underwent diurnal AC; the testicles were pushed up into the inguinal canal and secured in this position by a technical device: an athletic supporter provided with an orifice at the root of the penis allowing the penis and the scrotal skin to be exteriorized. With the device adjusted, the testicles were kept in inguinal position every day during waking hours.

Received January 22, 1986; revised and accepted September 15, 1986.
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At the outset, the volunteers gave their consent for at least 6 months of experimentation but were free to continue as long as they wished. They undertook to have bimonthly surveillance throughout the experiment and the following year. Of the 19 volunteers who undertook the experiment, 4 are still involved, and 15 have dropped out with exposure times ranging between 6 and 24 months. Among the latter, 2 have left the region, and contact has not been maintained. The length of individual follow-up studies available at the time of analysis of results varies from 6 to 18 months. Because of the variations in exposure and follow-up times, the number of cases is not constant throughout the study and is indicated for each period in the tables and figures. All 19 volunteers were studied for 6 months before the experiment and during at least 6 months of AC. Except for one exclusion for azoospermia at the eighth month of experimenting, dropping out was not a result of medical grounds, discomfort, or pain. Of the 15 dropouts, 13 underwent regular follow-up for periods ranging from 6 to 18 months, while the 2 others dropped out of sight. The azoospermic man remained so for two months after discontinuing the experiment, but his values then recovered to the level of the other volunteers. In this case, a morphologic study was therefore possible only from the second period following treatment.

SPERM EXAMINATION

The morphologic study involved 100 spermatozoa per sample, studied under optical microscope after fixation and staining (Papanicolaou). The detailed study of the abnormal forms was undertaken according to David et al.,[10] except that heads showing lysis were not recorded. The classification of abnormalities is (1) head: elongated, thin, microcephalic, macrocephalic, duplicate, and irregular; (2) tail: cytoplasmic remains, bent, absent, short, coiled, and duplicate. Each abnormality was evaluated separately as a percentage; abnormalities have been collected with respect to localization, head or tail, with abnormalities of the tail further divided into abnormalities of the middle and of the principal pieces. All sperm examinations were performed by the same examiner; the variation coefficients of measurements repeated on a given sample by the same operator were between 5% and 10% according to abnormality types.

STATISTICAL ANALYSIS

Each man was used as his own control, and for each parameter mean values for each period were compared with each of the three baseline means by use of Wilcoxon rank test. For minimization of possible bias due to baseline fluctuations, differences were deemed significant only if found with regard to each of the three baseline means for the treatment period and to at least one of the three baseline means for the period after treatment.

RESULTS

HEAT EXPOSURE PERIOD

Sperm Count and Motility

The spermatogenesis of the 19 men involved in the current study was appreciably reduced. Mean sperm count and motility, initially 89 $\times$ 10^6 spermatozoa/ml and 67%, respectively, dropped significantly as early as the second month of AC; respective values ranged between 5 $\times$ 10^6 and 18 $\times$ 10^6 spermatozoa/ml and 18% to 36% from the fourth to the twenty-fourth month of exposition.

Abnormally Shaped Spermatozoa

The mean percentage of abnormally shaped spermatozoa (Fig. 1), initially < 30% rose significantly and regularly from the second to the tenth month and then settled at about 50%.

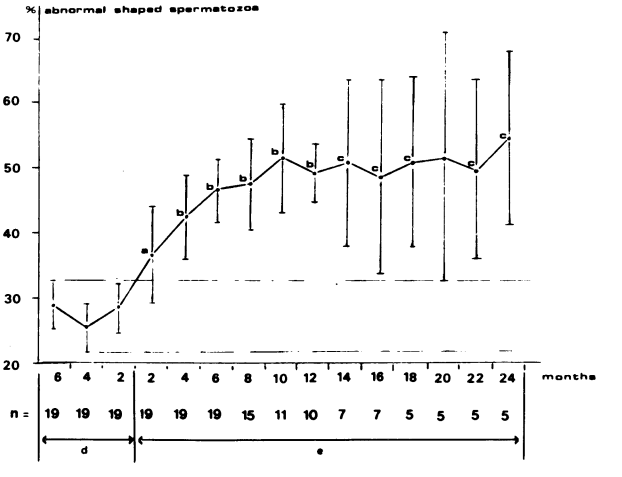

Figure 1
Abnormally shaped spermatozoa before and during AC. Values are mean percentages and 95% confidence limit of means (2 SE); a, $P < 0.01$; b, $P < 0.001$; c, $P < 0.05$; d, baseline study; e, AC experiment.

Table 1. *Mean Percentages of Spermatozoa Abnormalities Modified by AC*

Period	No. of men	Head			Bent tail
		Elongated	Thin	Irregular	
		%	%	%	%
6th month before AC	19	1.0	4.3	2.1	5.4
4th month before AC	19	1.3	3.9	1.9	5.0
2nd month before AC	19	2.2	4.2	2.3	6.2
2nd month during AC	19	5.1[a]	5.9	3.2	9.5[a]
4th month during AC	19	6.2[a]	9.6[b]	3.1	10.7[b]
6th month during AC	19	8.9[b]	9.1[a]	3.4[a]	11.4[b]
8th month during AC	15	10.1[b]	10.1[b]	4.3[a]	11.8[b]
10th month during AC	11	9.9[a]	9.9[a]	4.4[a]	11.8[b]
12th month during AC	10	7.1	15.3[b]	3.7	11.6[b]
14th month during AC	7	6.9	10.9[a]	4.0	13.3[a]
16th month during AC	5	4.2	11.2	5.4	13.2
18th month during AC	5	5.4	11.4[a]	3.8	12.0
20th month during AC	5	5.0	11.2	4.2	10.4
22nd month during AC	5	5.6	10.8	2.4	9.6
24th month during AC	5	8.4[a]	11.0	2.2	10.8

[a] $P < 0.05$; comparisons with each of the three baseline means (6th, 4th, and 2nd months before AC).
[b] $P < 0.01$; comparisons with each of the three baseline means (6th, 4th, and 2nd months before AC).

Different Types of Abnormalities

A statistically significant and regular increase of elongated heads was seen up to the tenth month (Table 1). Thin heads appeared to increase significantly up to the twelfth month and then remained relatively stable throughout the 24 months of experimentation. The percentage of irregular heads increased moderately. Microcephalic, macrocephalic, and duplicate heads, respectively 3%, 1%, and 1% before the onset of the experiment, remained constant throughout. With regard to the middle piece of the tail, only the percentage increase of bent tails was found to be statistically significant and relatively constant from the fourth to the twenty-fourth month (Table 1). Other abnormalities of the middle and principle pieces of the tail showed no significant variations (cytoplasmic remains, 4%; duplicate tails, 0.5%; no tail, 0.5%; short tails, 0.5%; and coiled tails, 5%).

Taken together (Table 2), the increase in head abnormalities was nearly threefold, abnormalities of the middle piece of the tail doubled, and abnormalities of the principal piece of the tail remained constant at 6.5%.

PERIOD AFTER AC

Mean percentage of abnormally shaped spermatozoa (Fig. 2) remained significantly higher than baseline ones up to the sixth month after treatment.

When the various types of abnormalities are considered, it is evident that they evolve differently from one another (Table 3). Abnormalities of the principal piece of the tail remained constant throughout the period after treatment as during the treatment period. Abnormalities of the

Table 2. *Abnormalities of the Head and the Middle Piece of the Tail Before and During AC*

Period	No. of men	Mean percentages (± SE[a]) of abnormalities	
		Head	Middle piece of tail
6th month before AC	19	13.8 (1.8)	10.4 (2.4)
4th month before AC	19	11.9 (1.2)	8.6 (0.9)
2nd month before AC	19	15.0 (1.4)	9.5 (1.0)
2nd month during AC	19	20.6[b] (3.4)	14.0[c] (1.6)
4th month during AC	19	24.9[c] (3.5)	15.1[c] (1.3)
6th month during AC	19	30.0[d] (2.4)	16.2[c] (1.9)
8th month during AC	15	30.9[d] (3.6)	15.7[c] (1.4)
10th month during AC	11	33.0[c] (5.1)	16.8[c] (1.7)
12th month during AC	10	32.7[c] (1.9)	15.6[c] (1.6)
14th month during AC	7	32.0[b] (6.2)	17.1[b] (2.6)
16th month during AC	5	29.0 (7.3)	17.0 (3.5)
18th month during AC	5	30.4 (7.4)	16.8 (3.4)
20th month during AC	5	36.8 (12.2)	16.0 (3.6)
22nd month during AC	5	34.8[b] (7.0)	13.4 (2.0)
24th month during AC	5	37.0[b] (7.6)	15.2[b] (2.8)

[a] SE, standard error.
[b] $P < 0.05$; comparisons with each of the three baseline means.
[c] $P < 0.01$; comparisons with each of the three baseline means.
[d] $P < 0.001$; comparisons with each of the three baseline means.

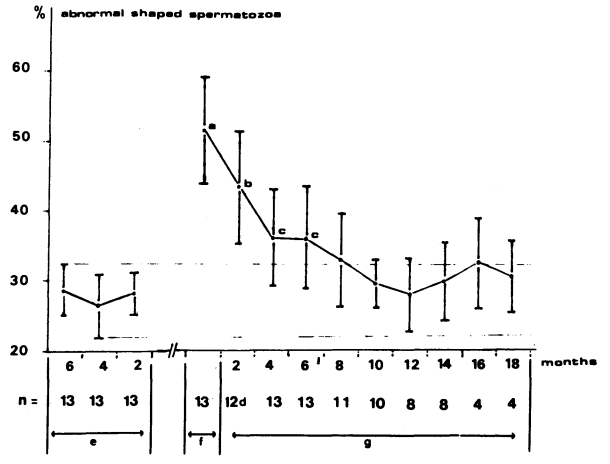

Figure 2
Abnormally shaped spermatozoa before and after AC. Values are mean percentages and 95% confidence limit of means (2 SE); a, $P < 0.001$; b, $P < 0.01$; c, $P < 0.05$; d, one man missing for azoospermia; e, baseline study; f, last 2 months of AC; g, after AC follow-up.

middle piece of the tail recovered to baseline values around the twelfth month (Table 4). Taken together, abnormalities of the head progressively decreased from the first 2 months of the period following treatment but remained higher than initial values 18 months after the end of experimentation (Table 3); this slightly increased rate of head abnormalities is due mainly to the mean percentages of elongated heads (Table 4).

The recovery of abnormally shaped spermatozoa was also studied with respect to the duration of hyperthermia, for exposure times ranging from 6 to 12 months (mean, 9 months) with eight volunteers and 24 months with the five other men. The values for both groups were similar before treatment and during the initial 12 months of exposure. Statistical analysis also failed to demonstrate any significant difference after treatment between the two groups either in mean percentages of abnormally shaped spermatozoa or in those of each type of abnormality.

Table 3. *Abnormalities of the Head and the Middle Piece of the Tail After AC*

Period	No. of men	Mean percentages (± SE[a]) of abnormalities	
		Head	Middle piece of tail
6th month before AC	13	14.5 (0.9)	10.6 (1.3)
4th month before AC	13	12.1 (1.4)	8.7 (1.1)
2nd month before AC	13	14.7 (1.5)	9.3 (1.2)
Last 2 months of AC	13	35.8[b] (4.0)	18.2[b] (1.7)
2nd month after AC	12[c]	28.9[b] (3.9)	15.0[d] (1.7)
4th month after AC	13	23.3[d] (3.1)	12.1[e] (1.9)
6th month after AC	13	24.3[d] (3.4)	11.0 (1.3)
8th month after AC	11	20.4[d] (2.9)	12.1[e] (1.8)
10th month after AC	10	17.3[e] (1.3)	10.2 (1.4)
12th month after AC	8	18.0[e] (1.3)	8.9 (1.2)
14th month after AC	8	19.9[e] (2.6)	9.8 (2.0)
16th month after AC	4	19.8[e] (2.2)	11.5[e] (2.0)
18th month after AC	4	20.5[e] (1.9)	7.8 (1.6)

[a]SE, standard error.
[b]$P < 0.001$.
[c]Values of one man missing because of azoospermia.
[d]$P < 0.01$.
[e]$P < 0.05$ with at least one of the three baseline values.

Table 4. *Mean Percentages of Spermatozoa Abnormalities After AC*

Period	No. of men	Head			Bent tail
		Elongated	Thin	Irregular	
6th month before AC	13	2.5	3.8	2.2	5.2
4th month before AC	13	1.5	4.1	1.9	4.9
2nd month before AC	13	2.2	4.0	2.1	6.1
Last 2 months of AC	13	11.4[a]	10.2[b]	3.0	18.8[a]
2nd month during AC	12[c]	9.6[a]	5.5	3.5[b]	9.2[a]
4th month during AC	13	5.7[a]	5.2	2.8	8.7[a]
6th month during AC	13	5.6[a]	5.9	2.6	8.1[b]
8th month during AC	11	5.4[b]	3.5	2.8	4.9
10th month during AC	10	3.9[b]	3.3	2.4	7.1[b]
12th month during AC	8	4.2[b]	3.0	2.7	5.8
14th month during AC	8	4.8[b]	3.0	3.9	6.4
16th month during AC	4	2.5	4.0	2.8	7.5
18th month during AC	4	5.2	3.8	2.8	4.8

[a]$P < 0.01$.
[b]$P < 0.05$ with at least one of the three baseline values.
[c]One man's values are missing because of azoospermia.

DISCUSSION

Involving five more cases, the current study is complementary to a previous work[9] and confirmed the reduction in sperm count and motility previously obtained. The current study shows that a slight and repeated increase in the environmental temperature of the testicles placed in inguinal position induced an increase in the percentage of abnormally shaped spermatozoa. Comparisons between the different hyperthermia experiments are difficult because of differences in source, intensity, and duration of heating. That increases in abnormal shapes were reported only in studies involving extended periods of local[4] or general[3] hyperthermia is noteworthy, although types of abnormality were not studied.

Three of the factors known to influence semen characteristics and liable to induce bias in semen studies are usually taken into account: individual variability, abstinence period, and seasonal influences. The baseline study performed during the 6 months before the onset of experimentation reflects the individual variability; in our results, we used the most unfavorable hypothesis (i.e., statistically significant differences were indicated only when present with each of the three baseline values for the treatment period and with at least one of the three baseline values for the period following treatment). Abstinence was found to affect all semen characteristics except morphologic features[11, 12]; thus variations recorded in the mean percentages of abnormally shaped spermatozoa cannot be explained by variations in the abstinence period. The volunteers have been involved in the experiment at various periods of the year so that no seasonal variations could explain the recorded modifications; moreover, as far as mean percentages of abnormally shaped spermatozoa are concerned, the amplitude of the observed variations is higher than those induced by seasonal variations.[13, 14]

The variations seen in our experiment appeared to be related to the effects of AC. Indeed, increases in the mean percentages of abnormally shaped spermatozoa and in abnormality types were seen after exposition, rising progressively throughout the experiment, whereas they began to disappear as soon as the exposition was interrupted. AC realized in this experiment induced a rise in environmental testicular temperature estimated at 1° to 2°C. Although other factors such as vascularization may have been modified by AC, the temperature increase seemed to account for modifications in the morphologic features of spermatozoa, because similar modifications were found in animals[15-19] and in man[3, 4] by studies where hyperthermia was induced without cryptorchidism. These modifications could be the result of an altered spermatozoa morphogenesis in seminiferous tubules.[20, 21]

Results from the period after treatment require particular attention for the appreciation of the innocuity and reversibility of the heat effect as a male contraceptive method. The mean percentages of abnormally shaped spermatozoa could lead to the consideration that the testicular function recovered its baseline status within an 8-month period. But the detailed study of the different types of abnormalities shows that abnormalities of the middle piece of the tail and of the head display values significantly higher than the initial ones 14 and 18 months after the end of the experiment, respectively. But such increases appear too moderate to play a significant role in fertilization[22-24]; this was confirmed by the fact that two volunteers displaying this moderate increase in abnormalities have fathered a desired child with their partner respectively 18 and 19 months after the end of AC.

Although our experimentation was designed to study a male contraceptive method, it is too early to assess its efficiency, but proof of reversibility has been given with regard to sperm count and motility[9] as well as to morphologic features. The deleterious effect of heat on human spermatogenesis, known as far as the sperm count is concerned, has also been shown to affect the morphologic features of spermatozoa by our study.

Acknowledgments. We wish to thank Ms. Charlotte Mondinat for her invaluable technical assistance and Mr. Frédéric Guéreau for his contribution in the preparation of the manuscript.

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