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Inhibiting effect of artificial cryptorchidism on spermatogenesis

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In order to provide a contraceptive method in man, an attempt at inhibition of spermatogenesis was made by means of thermogenesis induced by artificial cryptorchidism. This experimental protocol consists of pushing up the testicles into the inguinal canal and keeping them there, each day during waking hours, by means of an adapted athletic supporter. Fourteen men volunteered for this protocol during a 6- to 12-month period. They all had a normal semen analysis before starting the experiment. The total sperm count, the sperm count per milliliter, the motility, the motile sperm count per milliliter, and the total motile sperm count dropped significantly after the first or second month; they reached their lowest values after the sixth month and remained stable during the next 6 months. At that time the average values reached were a total sperm count of 12 to 34 × 10^6/ejaculate, a sperm count of 3 to 10 × 10^6/ml, a motility of 21% to 34%, a motile sperm count of 1 to 3 × 10^6/ml, and a total motile sperm count of 4 to 12 × 10^6/ejaculate. Fertil Steril 43:589, 1985

For over a century, scientists have studied the effects of testicular or scrotal hyperthermia on spermatogenesis, first in the animal[1, 2] and then in man. In man, the various experiments, always limited in duration to less than 3 months, have led to several essential conclusions. Febrile states are capable of reducing the number of spermatozoa through a direct effect of the temperature.[3] Scrotal hyperthermia induces a depression of the motility[4] and an increase in the percentage of abnormal forms of spermatozoa.[5] Moreover, the experiments of Robinson and Rock,[6] evaluating the effects of scrotal hyperthermia on spermatogenesis using various heat sources, have led them to make the following three remarks: "Fertility might be affected by a sustained yet relatively slight increase in the scrotal temperature." "There was strong evidence that the longer the treatment, the more the spermatogenesis could be depressed." "These results may have application to the development of a simple method of male fertility control." The originality of the present experiment lies in the use of the human body as a heat source over a long period (12 months), in order to provide a male contraceptive method.

MATERIALS AND METHODS

This study regroups the results of experimentation with 14 men over a 3-year period. These men were all volunteers and invented this method of testicular heating themselves.

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Among these 14 men, between 23 and 31 years of age, 2 were fathers. They all had a normal semen analysis before the experiment, the range of individual values observed being as follows: volume, 1.5 to 8.0 ml; sperm count, 25 to 125 $\times$ 10^6 spermatozoa/ml; total sperm count, 62 to 617 $\times$ 10^6/ejaculate; progressive motility in the first hour, 60% to 80%; total motile sperm count, 38 to 401 $\times$ 10^6 motile spermatozoa/ejaculate; and morphology, 60% to 95% of normal forms.

METHOD OF TESTICULAR EXPOSURE TO HEAT

The heat source is the human body. The testicles are pushed up into the inguinal canal and are kept there by a technical device: an adjusted athletic supporter with an orifice at the root of the penis allowing the man to exteriorize the penis and the scrotal skin. Once the perforated athletic supporter is in place, the inferior pole of the testicle so pushed up is located at the superior part of the root of the penis. The testicles are kept in their inguinal position each day during waking hours.

After explanations and demonstration, all of the volunteers succeeded in pushing their testicles into the inguinal canal.

EXPERIMENTATION PROTOCOL

At the beginning of the experiment, two semen examinations were performed. The average values of the two tests are considered as the reference values for each man. Then, after an adaptation period lasting 1 to 3 weeks, the perforated athletic supporter was worn for 12 months. The evolution of the semen parameters during this time was studied in 13 periods of 4 weeks each.

Among the 14 men, 7 followed the protocol throughout the entire year, the other 7 stopped at various times, between the 7th and the 12th periods. The reasons for stopping were nonmedical in six cases, but one man was excluded at the ninth period after 5 consecutive weeks of azoospermia (weekly control).

At the end of the experiment, a follow-up was performed for 12 months.

SPERM EXAMINATION

Sperm specimens are collected by masturbation after a 2- to 5-day abstinence period.[7] The collection is done either at the laboratory or at home; in this case, the sample is brought to the laboratory within 30 minutes. Each sample is examined within 1 hour, after being placed in the incubator at 37°C. All of the sperm examinations were done at the CECOS Midi-Pyrénées Laboratory by the same person.

The volume is measured by direct reading on the graduated container. The motility at 1 hour is read under an optical microscope; it is expressed as the percentage of progressive motility (sperm crossing the visual field of the microscope). Sperm counts are performed on a Mallassez cell (Rogo and Company, Arcueil, France) as follows: after dilution to the twentieth in a sodium bicarbonate-formol solution, an aliquot of the sperm sample is placed between the Mallassez cell and an overlying flat-surface slide; the so isolated volume corresponds to 1 mm^3. The percentage of normal forms is calculated on 100 spermatozoa examined under the optical microscope after fixation and coloration (Papanicolaou). The vitality is appreciated by eosin-nigrosin coloration of one drop of fresh sperm. The pH is measured with reaction paper.

In addition, three parameters are calculated from the previous measures: the total sperm count (volume of the ejaculate $\times$ sperm count per milliliter), the motile sperm count (sperm count $\times$ percentage motility), and the total motile sperm count (total sperm count $\times$ percentage motility).

STATISTICAL ANALYSIS

Two types of statistical comparisons are made: (1) for each parameter, the values of the various periods are compared with the initial value; (2) at each period, the percentages of variation of the various parameters are compared with each other. For all of these comparisons, we used Student's paired *t*-test, each subject being taken as his own control.

RESULTS

The results are given in real values in Table 1 (mean $\pm$ standard deviation [SD]) and as a percentage of the initial values in Figures 1 and 2.

During the experiment, the volume varied little; values lower than the initial values were observed from the first to the fifth period; however, this decrease is statistically significant only for the fourth period.

Table 1. *Characteristics of Semen Samples Before and During Artificial Cryptorchidism*

	Period (wks)													
	0 (Before)	1 (1–4)	2 (5–8)	3 (9–12)	4 (13–16)	5 (17–20)	6 (21–24)	7 (25–28)	8 (29–32)	9 (33–36)	10 (37–40)	11 (41–44)	12 (45–48)	13 (49–52)
No.	14	8	10	11	11	12	14	11	13	12	9	9	8	8
Volume (ml)														
Mean	3.6	2.7[a]	2.9[a]	2.7[a]	2.8[b]	2.9[a]	3.3[a]	3.3[a]	3.2[a]	3.3[a]	3.6[a]	3.1[a]	3.3[a]	3.1[a]
SD	1.2	0.8	0.6	0.7	1.3	1.1	1.1	1.6	1.0	1.5	2.3	1.1	0.8	1.4
Total sperm count (10^6/ejaculate)														
Mean	255	139[b]	120[c]	74[d]	54[d]	49[d]	62[d]	34[d]	33[d]	25[d]	12[c]	15[c]	19[c]	34[c]
SD	135	98	105	46	42	53	56	34	42	17	14	17	16	33
Sperm count (10^6/ml)														
Mean	72	49[a]	39[b]	27[d]	19[d]	14[d]	18[d]	10[d]	10[d]	8[d]	3[d]	5[d]	6[d]	10[d]
SD	30	28	32	18	16	12	14	8	10	7	2	4	5	9
Motility (%)														
Mean	68	61[c]	48[c]	46[c]	37[d]	32[d]	41[d]	31[d]	23[d]	21[d]	24[d]	28[d]	34[d]	29[d]
SD	7	7	12	17	18	17	14	16	12	15	15	11	15	10
Motile sperm count (10^6/ml)														
Mean	51	30[b]	21[b]	13[d]	10[d]	6[d]	8[d]	3[d]	3[d]	2[d]	1[d]	1[d]	2[d]	3[c]
SD	24	16	18	13	10	6	7	3	4	4	1	1	2	4
Total motile sperm count (10^6/ejaculate)														
Mean	174	83[c]	63[c]	38[d]	25[d]	21[d]	28[d]	10[d]	10[d]	7[d]	4[c]	4[c]	7[c]	12[c]
SD	89	52	58	27	24	27	27	11	18	8	5	4	6	12
No.	14	8	10	10	10	10	12	9	11	11	6	7	7	7
Normal forms[e] (%)														
Mean	74	68[a]	66[b]	64[c]	60[d]	58[d]	58[d]	56[c]	59[d]	54[d]	59[b]	51[d]	47[d]	50[c]
SD	8	9	14	9	5	7	6	8	11	10	12	13	10	14

[a]Not significant.
[b]$P < 0.05$.
[c]$P < 0.01$.
[d]$P < 0.001$.
[e]Only in men with a sperm count $\geq 1 \times 10^6$/ml.

The total sperm count, the sperm count per milliliter, the motility, the motile sperm count, and the total motile sperm count dropped significantly after the first or second period, and the lowest values were seen at periods 7 to 13. At that time, the average values reached were as follows: a total sperm count of 12 to 34 $\times$ 10^6/ejaculate, a sperm count of 3 to 10 $\times$ 10^6/ml, a motility of 21% to 34%, a motile sperm count of 1 to 3 $\times$ 10^6/ml, and a total motile sperm count of 4 to 12 $\times$ 10^6/ejaculate. From the 7th to the 13th period, for each of these five parameters, the statistical analysis does not allow us to identify any one period with values significantly lower than those of the others.

The percentage of normal forms decreased very progressively from the second period on, but less than the previous parameters. At the end of the experiment, it was still at 50%.

Figure 1 shows the evolution of the total sperm count, the motility, the percentage of normal forms, and the total motile sperm count, expressed as a percentage of initial values. The percentage of normal forms is the parameter showing the most moderate and regular decrease; the most important drop was 35%. The total sperm count shows a very important decrease: the lowest values, seen from the 7th to the 13th period (during 28 weeks), correspond to a decrease of 84% to 95%. The motility occupies an intermediate position between these two parameters: its decrease is slower and less than that of the total sperm count; the decrease is over 50% from the seventh period on, whereas that of the total sperm count has reached that decrease by the third period; statistical comparisons have shown that from the third period on, the depression in total sperm count was significantly greater than that of the motility. The most important depression is shown by the total motile sperm count: the lowest values, seen at periods 7 to 13, represent a drop of 92% to 98%; statistical comparisons have shown that the depression in total motile sperm count was significantly greater than that of total sperm count, from the 2nd to the 12th period ($P < 0.05$).

Figure 2 shows that the decrease in sperm count and motile sperm count follows a parallel trend throughout the experimentation period; however, the decrease in the motile sperm count is significantly greater ($P < 0.05$) for all periods

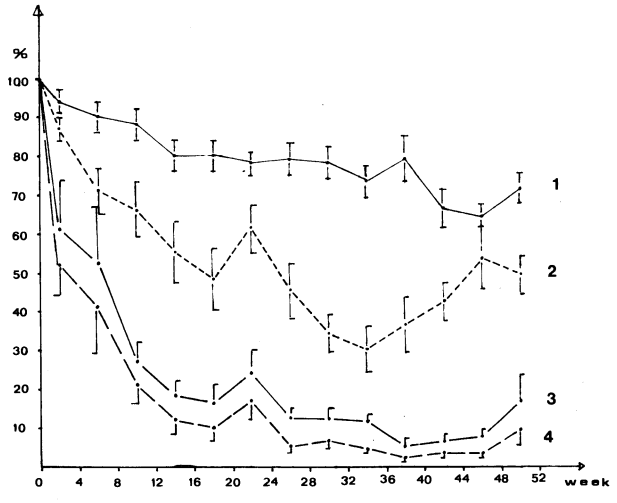

Figure 1
(1) Evolution of the percentage of normal forms, (2) motility, (3) total sperm count, and (4) total motile sperm count expressed as a percentage of initial values.

except for the first and fourth periods. The lowest values observed between the 7th and 13th periods are 85% to 95% for the sperm count and 92% to 98% for the motile sperm count.

Of all the parameters studied, the motile sperm count and the total motile sperm count are the most depressed and the most constant in depression.

No significant changes in pH were observed throughout the experiment (7.4 ± 0.1 before; 7.4 ± 0.2 to 7.5 ± 0.1 during), and vitality remained unaltered: 92% ± 4% of live sperm before, and 90% ± 4% to 92% ± 3% during the experiment.

An extreme case of azoospermia was observed in one man: at the beginning, his total sperm count was 110×10^6/ejaculate; it became $< 1 \times 10^6$ from the 18th to 25th week (weekly sperm control) and reached 0 from the 26th to the 29th week, i.e., during 4 successive weeks. To our knowledge, there is no publication in the literature describing azoospermia induced by testicular hyperthermia in man. Without data allowing affirmation that this azoospermia may be reversible, it was found preferable to stop the experiment with this man. He remained azoospermic until the third week after stopping the artificial cryptorchidism, and he had a total sperm count of 3×10^6/ejaculate by the fifth week, 143×10^6 by the seventh week, and 231×10^6 by the ninth week.

Ten men who stopped the experiment (after at least 6 months of hyperthermia) were followed for 5 to 12 months (Table 2). All the parameters studied returned to the initial range of values within a 6- to 8-month period. A more complete and detailed study of the recovery phase will be published later.

DISCUSSION

Among the published articles concerning the effects of hyperthermia on spermatogenesis, two[3, 5] describe methods elevating total body temperature ("artificial fever" and sauna) and four[4, 6, 8, 9] report various methods of local scrotal heating (hot water bath, scrotal insulation, lamp). Even though the principles and the length of experimentation vary in these studies and the mechanisms implied seem different, the results obtained on the whole are quite similar and, for the most part, are confirmed in our work.

In all of the studies performed in man, two basic physiologic data are used. First, testicular temperature is always lower than body temperature. The mean difference between them varies according to author[6, 8-14] from 1.4°C to 4.4°C; the discrepancy of results seems essentially related to the conditions in which the temperatures are measured.[15] Second, this testicular "hypothermia" is the result of two processes: vascular countercurrent thermal exchanges[16] and the thermoregulator mechanisms of the scrotum,[17] inducing a body-testis thermal gradient. The displacement of the testicles used in this experiment eliminates the thermoregulator effects of the scrotum[17] and modifies their thermal environment.[13, 14] Be-

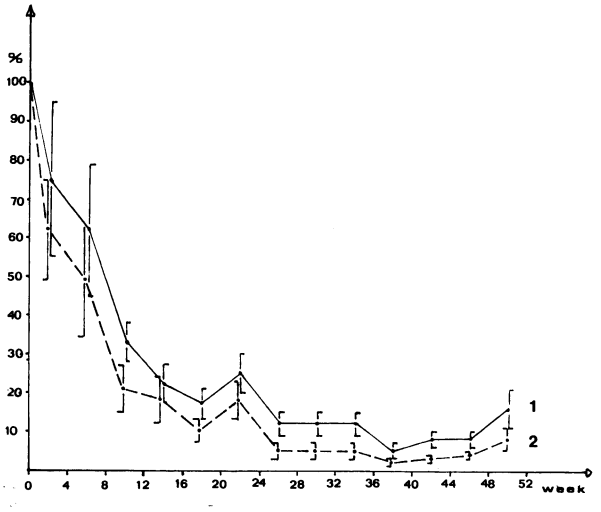

Figure 2
(1) Evolution of the sperm count and (2) motile sperm count expressed as a percentage of initial values.

Table 2. *Characteristics of Semen Samples After Artificial Cryptorchidism*

	Last period of cryptorchidism	Period (wks)													Before cryptorchidism
		1 (1–4)	2 (5–8)	3 (9–12)	4 (13–16)	5 (17–20)	6 (21–24)	7 (25–28)	8 (29–32)	9 (33–36)	10 (37–40)	11 (41–44)	12 (45–48)	13 (49–52)	
No.	10	6	8	7	9	10	7	6	6	6	4	4	5	5	10
Sperm count (10^6/ml)															
Mean	9	12[a]	55	45	67	63	40	70	100	80	119	64	73	88	75
SD	11	10	21	22	41	38	22	50	50	40	65	36	50	48	30
Motility (%)															
Mean	23	33[b]	51[b]	56[b]	60[c]	61	60	64	67	67	70	65	69	64	70
SD	16	17	6	10	9	11	9	4	3	4	8	9	12	15	8
No.	7	5	7	7	8	9	6	6	6	6	3	4	5	5	10
Normal forms[d] (%)															
Mean	50	55[c]	67	65[c]	66[c]	67[c]	67	68	69	72	77	68	72	76	75
SD	6	8	7	10	6	5	15	10	3	5	14	8	9	8	7

[a] $P < 0.001$
[b] $P < 0.01$
[c] $P < 0.05$
} comparison with values observed before cryptorchidism.

[d] Only in men with a sperm count $\geq 1 \times 10^6$/ml.

cause the scrotal temperature is 0.5° to 1°C lower than the testicular temperature and this latter is 1° to 1.5°C lower than the temperature in the inguinal canal, one can estimate at 1° to 2°C the temperature increase produced in the testicular environment by this technique.

Our results show that this slight temperature increase, sustained during waking hours and repeated every day, is sufficient to bring about an important inhibition of spermatogenesis. After 12 weeks of such a slight temperature increase, men become oligoasthenozoospermic (mean sperm count $< 20 \times 10^6$/ml; motility, 20% to 40%) and so remain at least throughout the period of artificial cryptorchidism. These effects, associated with an increased percentage of abnormal forms, are to be compared with the alterations of sperm parameters often observed with a varicocele; the role of hyperthermia in this case seems demonstrated by the results of chronic hypothermic treatment in patients whose varicocele is associated with a scrotal temperature above normal.[18] Moreover, these results underline the necessity to pay attention to thermic factors in men consulting for infertility and having oligoasthenozoospermia; in these cases, searching for external factors inducing hyperthermia and scrotal or testicular temperature recordings should be done routinely. An effort to standarize the ways and areas of such measurements seems important.

Once the psychologic barriers of accepting the experimental protocol are overcome, it is easy to carry out and offers complete autonomy for the man. Throughout the 52 weeks, no health problems appeared in relation to this experimental study; and after the end of the experimentation, sperm parameters progressively returned to normal values in a 6- to 8-month period.

This method still begin experimental, the only possible appreciation of the contraceptive effects obtained lies in the alterations in spermatogenesis. From this point of view, it must be noted that, before experimentation, sperm analysis of these men placed them all into a class of "normal fertility," whereas from the 13th week of testicular hyperthermia their mean sperm parameters put them into a class of "hypofertility." In addition, from the 25th to the 52nd week, the mean total motile sperm count varied between 2 and 12 $\times$ 10^6/ejaculate; and it has been demonstrated that there is a drop in the percentage of pregnancies for men having $< 12.5 \times 10^6$ motile spermatozoa/ejaculate.[19] However, hypofertility does not mean infertility; therefore, a more critical appreciation of the fertilizing capacity of sperm is in progress with another group of men following the same protocol, using various approaches, including the sperm penetration assay.[20] It seems of utmost importance to await these results before planning to evaluate the real contraceptive effect of this method.

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