Seasonal Changes in Human Sperm Chromatin Condensation

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Submitted: November 3, 2000
Accepted: February 28, 2001

Purpose: *The aim of this study was to investigate possible seasonal changes in human sperm parameters, especially chromatin condensation.*
Method: *In a first run, 3155 patients attending the andrological outpatient clinic at the Centre of Dermatology and Andrology at Justus Liebig University, Giessen, Germany, from January 1992 to October 1995 were examined for sperm count, motility, vitality, and chromatin condensation.*
Results: *The respective results were correlated according to season. Significant seasonal changes were observed in chromatin condensation and sperm count, with mean maximum values (for chromatin condensation and sperm count) of 86.24% aniline blue-negative spermatozoa in January and 68.75 $\times$ 10^6 mL^{-1} in April. To confirm the observation of seasonal changes in sperm chromatin condensation in Germany on the Southern Hemisphere, 179 patients attending the Reproductive Biology Unit at Tygerberg Hospital, Tygerberg, South Africa, were examined by means of the aniline blue stain from April 1999 to April 2000. For chromatin condensation, a significant seasonal change shifted by 4–5 months was observed on the Southern Hemisphere. However, no seasonal variations could be found for the sperm count.*
Conclusions: *Our results clearly demonstrate seasonal changes in sperm count and chromatin condensation. In contrast, no circannual relation was observed for motility and vitality.*

KEY WORDS: chromatin condensation; human spermatozoa; seasonal changes; sperm count.

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INTRODUCTION

Among the animal kingdom most animals, including mammals, exhibit a seasonal rhythm in their reproductive phases. Only a few species such as guinea pig, pig, laboratory rat, and among the primates the human, show no seasonal pattern of reproduction (1). However, there are a number of clear indications that seasonal rhythms also play a role in human reproduction. This is obvious by seasonal variations in the birth rates, which were proved by epidemiological studies (2,3). On the other hand, circannual rhythms were observed in naturally conceiving women (4). In addition, it has been shown that an increased incidence of endometrium hyperplasia might be correlated with more frequent anovulatory cycles during the winter period (5). Finally, variations in oocyte quality and ovulation (6,7), together with considerable fluctuations in sperm quality, are thought to be responsible for poor results of assisted reproduction (8,9).

In males, highest plasma concentrations of the hormones LH, testosterone, and estradiol appear in autumn, whereas FSH and 20α-dihydroprogesterone reach their highest levels in summer (10). With regard to sperm concentration, motility, and vitality, contradictory results have been published. While Mortimer *et al.* (11) did not show significant seasonal variations of these parameters, results obtained by Tjoa *et al.* (12) and Saint Pol *et al.* (13) clearly emphasize such a seasonality effect.

Our retrospective study was aimed at clarifying whether seasonal influences on human sperm parameters such as sperm count, motility, and vitality may play a role in the central European area. In addition, the question was addressed as to whether chromatin condensation as functional sperm parameter might be subject to circannual rhythms.

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MATERIAL AND METHODS

This retrospective study included a total of 2,180 patients attending the andrological outpatient clinic of the Centre of Dermatology and Andrology at Justus Liebig University Giessen, Germany. From 1 January 1992 to 31 October 1995, chromatin condensation and spermiogram parameters (sperm count, motility, and vitality) were investigated and correlated with the season.

Sperm count, motility (global motility: WHO A–C; progressive motility: WHO A), and vitality were analyzed according to WHO criteria (14). Quality of sperm chromatin condensation was tested by means of the aniline blue stain according to Terquem and Dadoune (15). Spermatozoa showing strong (++) and very strong (+++) stain with aniline blue were regarded as aniline blue-positive. Those staining only weakly (+) or not at all (0) were regarded as aniline blue-negative.

To gain a closer insight into the seasonality of sperm functions we also examined spermiogram parameters and chromatin condensation on the Southern Hemisphere. For this purpose, a total of 179 ejaculates from patients attending the Reproductive Biology Unit of Tygerberg Hospital, Tygerberg, South Africa, from April 1999 to April 2000 were analyzed.

For evaluation of these data, single values for each parameter were grouped on a monthly basis, the arithmetic mean was calculated and plotted against the calendar months. In addition, the percentage of aniline blue-positive and -negative spermatozoa was correlated with data of the monthly mean maximum day temperature, mean day temperature, and a calculated mean perceived day temperature. The weather data were obtained from the German Weather Service, Offenbach, Germany, and reflected the weather conditions of Giessen during the mentioned period.

Statistical calculations were carried out with MedCalc 2.20, MedCalc Software, Mariakerke, Belgium.

RESULTS

In the German part of the study, sperm count (Figs. 1 and 2a) and chromatin condensation (Figs. 3 and 4) were clearly subject to seasonal changes. For the sperm count this is obvious by comparison of the seasons. In spring time, the mean sperm count showed a maximum with 66.7×10^6 mL^{-1} (median: 41.2×10^6 mL^{-1}) (Fig. 2a) with a maximum value (68.75×10^6 mL^{-1}) observed in April. In contrast, the lowest sperm count with 52.25×10^6 mL^{-1} (median: 30.8×10^6 mL^{-1}) was found in autumn. The difference between spring and autumn was highly significant ($p = .0008$). The lowest sperm count per month was observed in November (46.0×10^6 mL^{-1}). An intermediate position was taken by summer and winter periods with 59.68×10^6 and 58.63×10^6 mL^{-1}, respectively, which did not differ ($p = .4321$). However, the differences between spring and summer, and spring and winter, were significant ($p = .0013$; $p = .0136$). In contrast, on the Southern Hemisphere no changes were found between spring ($n = 58$;

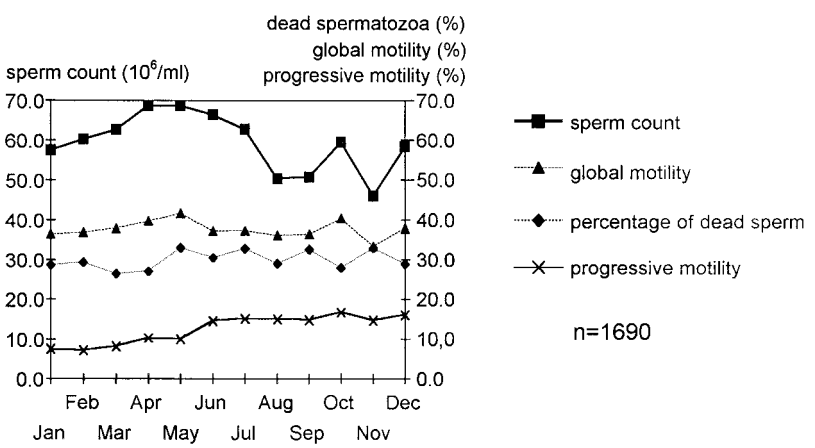

Fig. 1. Relationship between season and different spermiogram parameters in 1690 patients. The sperm count peaks in April and has its lowest values in autumn. For the other parameters, i.e., global motility, progressive motility, and percentage of dead sperm, no seasonal changes can be seen.

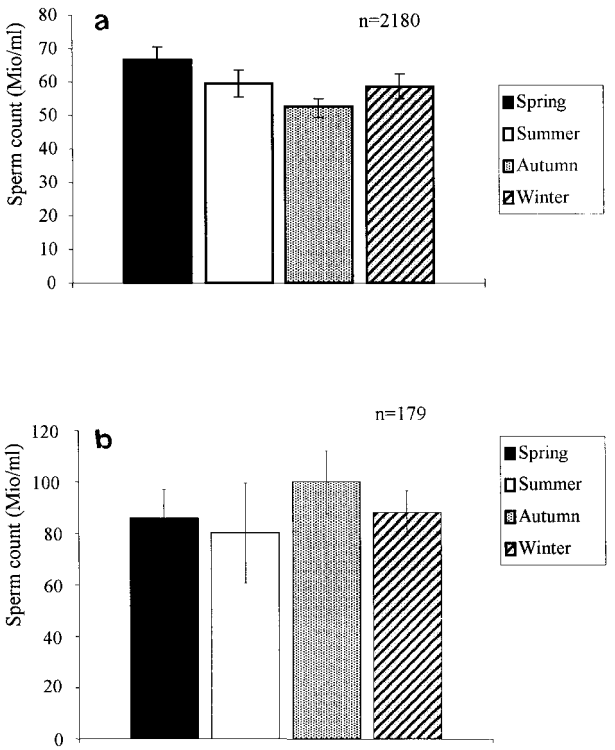

Fig. 2. Relationship between season and sperm count in the ejaculate in (a) Germany and (b) South Africa. In Germany (a), the highest sperm count can be found with 66.70×10^6 mL^{-1} in spring, while the lowest with 52.25×10^6 mL^{-1} appear in autumn. Summer (59.68×10^6 mL^{-1}) and winter (58.63×10^6 mL^{-1}) take an intermediate position. The sperm count in spring is significantly higher than in summer ($p = .0013$), autumn ($p = .0008$), and winter ($p = .0136$). In South Africa (b), no seasonal changes in sperm count can be seen. Sperm counts in different seasons do not differ.

mean: 87.6×10^6 mL^{-1}) and autumn ($n = 50$; mean: 108.9×10^6 mL^{-1}) ($p = .2138$).

A distinct correlation with the seasonal temperature was observed for the chromatin condensation (Fig. 3). In July, the month with the highest temperature, the mean percentage of aniline blue-positive spermatozoa, i.e., those showing disturbed chromatin condensation, was highest with 34.58%. In contrast, the highest percentage of aniline blue-negative spermatozoa was found in January (86.24%). The 2-month mean for aniline blue-positive sperm heads taken from January/February ($n = 373$; mean: 16.03%) and from August/September ($n = 396$; mean: 31.39%) differed highly significantly ($p = .0001$). This rhythm appeared throughout the observation period from January 1992 to October 1995 (Fig. 4).

Comparing the mean values of the northern and the southern hemispheres, a shift of 4–5 months was observed (Fig. 5). While the lowest number of aniline blue-negative spermatozoa in Germany occurred between July and August, the nadir was seen during February/March in South Africa. The 2-month mean for aniline blue-positive sperm heads from January/February ($n = 32$; mean: 21.43%), and from August/September ($n = 25$; mean: 13.84%) also differed highly significantly ($p = .0014$). A possible influence of higher summer temperature on the sperm staining quality was tested and could be excluded as cause of these rhythmic changes in chromatin condensation (data not shown).

In contrast to chromatin condensation (Figs. 3 and 4) and sperm count (Fig. 2a), no seasonal changes were found for the parameters motility (global and progressive motility) and vitality on the northern hemisphere (Fig. 1). On the Southern Hemisphere, however, clear seasonal changes were only observed for chromatin condensation (Fig. 5), while no differences were found for sperm count (Fig. 2b) and motility (Fig. 6).

DISCUSSION

The results of this study show no seasonal dependence of human global or progressive sperm motility and the percentage of dead spermatozoa. In contrast, sperm count as determined for patients in Germany was clearly subject to a seasonal rhythm, with the maximum number of spermatozoa occurring in springtime. This study confirms results by Tjoa *et al.* (12) and Saint Pol *et al.* (13), who also observed significantly higher sperm counts in spring than in autumn. Sperm counts obtained from patients in Cape Town, South Africa, however, revealed no seasonal changes. On the other hand, seeing that in Germany a highly significant difference for the sperm count was observed between spring and autumn but not on the Southern Hemisphere, might indicate a higher variation in this parameter. Then the relatively low number of patients in Cape Town (179 vs. 2180 in Germany) could be a possible reason for this obvious difference between the two locations.

The reasons for seasonality of sperm count on the northern hemisphere, especially the molecular relationship of this phenomenon, remain speculative. A direct influence of spermatogenesis by the hormones that regulate this process (10,16) cannot be ruled out, all the more since the peaks of testosterone, LH, and estradiol occurred during the last quarter of the year

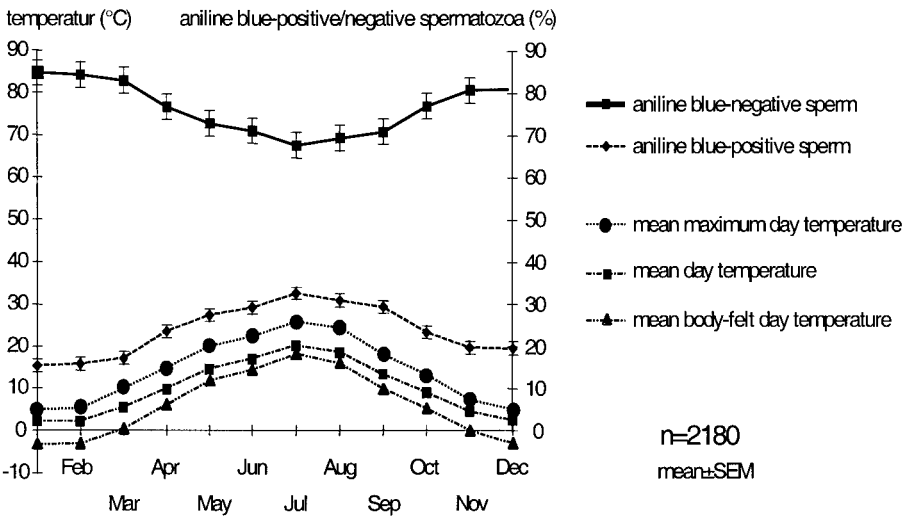

Fig. 3. Relationship between the percentage of aniline blue-positive and aniline blue-negative sperm with different temperature parameters in Germany. The percentage of aniline blue-positive spermatozoa directly follow the respective temperature curves and show its maximum in July. The curve for aniline blue-negative spermatozoa shows an inverse run.

in the northern hemisphere. Considering the whole period of a spermatogenesis cycle including epididymal sperm maturation lasts almost 3 months, seasonal regulation of human spermatogenesis with highest sperm count in spring seems likely. On the other hand, a direct photoperiodic control of spermatogenesis might also be possible. Melatonin, which controls the seasonal phase of reproduction in many mammalian species (17), may be involved in this connection. The antigonadal effect of melatonin is well known (18). However, it remains to be confirmed that melatonin has an influence on human LH secretion similarly to that in the rat (19).

In this report, it is shown for the first time that a functional sperm parameter, chromatin condensation, had a significant circannual rhythm and was correlated with temperature changes in the course of the year. In July/August, when highest temperatures are reached in Germany, the percentage of aniline blue-negative spermatozoa, indicating good nuclear maturity, show a nadir. In contrast, sperm nuclear maturity, evidenced by a high percentage

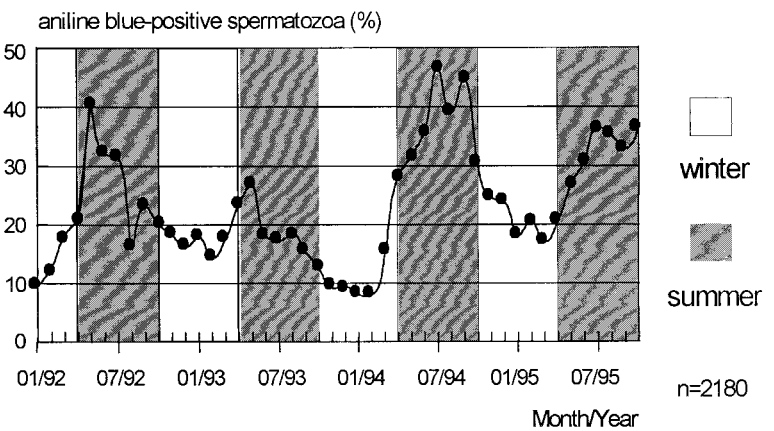

Fig. 4. Relationship between chromatin condensation as determined by the aniline blue stain and season during the time period between January 1992 and October 1995 for Giessen, Germany. The figure shows the regularly peaking percentage of aniline blue-positive spermatozoa during summer.

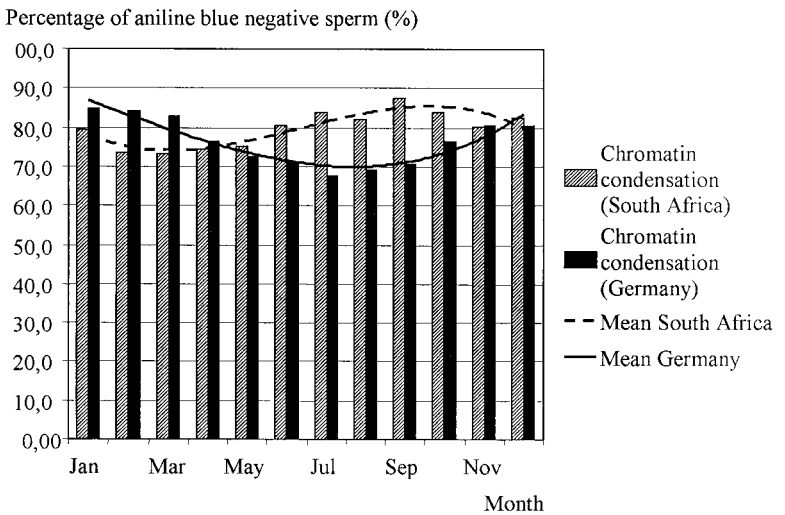

Fig. 5. Relationship between chromatin condensation as determined by aniline blue stain and season on the Northern Hemisphere ($n = 2180$; Giessen, Germany) and on the Southern Hemisphere ($n = 179$; Cape Town, South Africa). A significant seasonal change in human sperm nuclear chromatin condensation can be seen on both hemispheres. The minima of the percentage of sperm with good chromatin condensation (aniline blue-negative sperm) can be seen in July/August (Germany) and February/March (South Africa), respectively. There is obviously a shift of about 4–5 months between both hemispheres.

of aniline blue-positive sperm heads, was highest in December/January when the temperature was low. On the Southern Hemisphere (Cape Town, South Africa), a similar course for chromatin condensation was observed throughout the year, but with a shift of 4–5 months, the nadir of good chromatin condensation occurring in February/March (summer time on the Southern Hemisphere). This difference in sperm chromatin condensation between summer and winter was highly significant for both hemispheres.

Considering our data of mean temperature in Germany, these results would imply that replacement

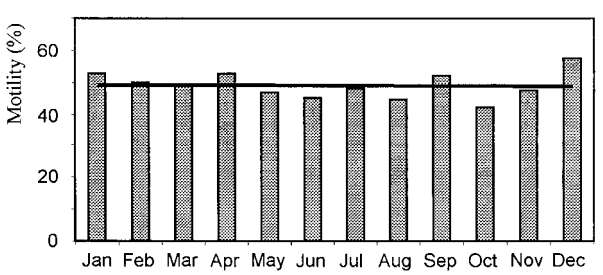

Fig. 6. Relationship between season and human sperm motility in South Africa. The black line indicates a trend line and shows that there is no seasonal change in motility ($n = 179$).

of histones by cysteine-rich protamines, which occurs during late spermatid stages (20), might be temperature-sensitive, as are because the Sertoli cells that control spermatogenesis are temperature-sensitive in specific functions, e.g., formation of tight junctions (21). An essential prerequisite for this hypothesis, however, would be that testicular temperature would be slightly increased because of heat congestion during the hot season despite light clothing. This idea remains to be proven.

On the other hand, this study could not distinguish between the hottest month (July) and the month with the longest daylight (June) in central Europe. Therefore, it might also be possible that the changes in chromatin condensation during the course of the year depend on the photoperiod. In animal models, melatonin had both anti-gonadal and fertility-supporting effects. Hence, a clear statement on the actual seasonal influence on human sperm chromatin condensation cannot be given at the moment.

Obvious seasonal changes in the percentage of nuclearly matured sperm in the course of the year should be taken into account when patients are counselled for infertility and tested for chromatin condensation in the cold season. Chromatin condensation has repeatedly proved to be predictive of fertilization

in vitro (22,23). If sperm nuclear maturity is tested in the cold season for IVF treatment to be performed in the hot season, this parameter might be low-ranged and therefore be a cause of failed fertilization. Knowledge of this fact is even more important because for sperm separation by the swim-up technique, which is commonly used prior to IVF, a significant decrease in the percentage of aniline blue-negative spermatozoa, i.e., those with normally chromatin condensation (24), occurs. In such cases, sperm separation by means of glass wool filtration, which increases the percentage of aniline blue-negative spermatozoa (24), might be beneficial.

In conclusion, from our data it is obvious that there is a significant seasonal influence not only on human sperm count, but also on chromatin condensation as a functional sperm parameter, with a shift of approximately 6 months between the northern and southern hemispheres. This observation confirms the first report by Sánchez *et al.* (25) who found a tendency towards increased quality of human sperm chromatin condensation in the South American late autumn from April to June. Since both parameters are of considerable importance for the outcome of assisted reproductive techniques, seasonal influences on sperm parameters are clinically significant and should be taken into account when counselling patients. To further investigate the reasons for this phenomenon, especially of the seasonal rhythm of chromatin condensation, a multicenter study of fertility centers on the Southern Hemisphere (Chile and South Africa) has been initiated.

ACKNOWLEDGMENTS

The authors thank Mrs. A. Hanschke (Department of Dermatology and Andrology, JLU Giessen), Dr. K. Coetzee, M.-L. Windt-De Beer, K. Smith, and E. Erasmus (IVF laboratory at Tygerberg Hospital) for skillful technical assistance and Mrs. G. Scharfe for linguistic review.

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