Scanning Microscopy

Volume 1 | Number 3 Article 38

5-28-1987

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Scanning Microscopy, Vol. 1, No. 3, 1987 (Pages 1241-1247)
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

0891-7035/87$3.00+.00

SPERM NUCLEAR ZINC, CHROMATIN STABILITY, AND MALE FERTILITY

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(Received for publication December 01, 1986, and in revised form May 28, 1987)

Abstract

Zinc excreted from the human prostate secures a high content of zinc in the sperm nucleus and contributes to the stability of the quaternary structure of the chromatin. After ejaculation, in vitro, a second type of stability, most probably involving disulfide-bridge crosslinks, supersedes the zinc-dependent stability. Normally, the nucleus of the ejaculated spermatozoon remains stable, i.e., it does not decondense when exposed to a detergent (e.g., sodium dodecyl sulfate - SDS), whereas a spermatozoon which has been exposed to a zinc-chelating medium becomes destabilized and decondenses in SDS. Spontaneous decondensation in SDS, i.e., without prior treatment with zinc-chelators, occurs among many spermatozoa from some infertile men, especially men with impaired secretory function of the prostate. This indicates that spontaneously decondensing spermatozoa have an inadequate content of zinc at ejaculation. Here, zinc in the sperm nucleus and chromatin stability was studied in semen samples from a group of men living in marriages with hitherto unexplained cause for infertility, and a group of fertile donors, who participated in an insemination program. Sperm nuclear zinc was studied with X-ray microanalysis and chromatin stability was assessed as percentage spermatozoa with stable sperm heads after exposure to SDS. Fertile donors had higher content of zinc in the sperm nuclei and had also higher proportions spermatozoa with a stabilized chromatin, than had the men living in infertile marriages. A positive rank-correlation was found between percentage of stable spermatozoa and sperm nuclear zinc. Zinc may stabilize the chromatin by forming salt-bridges between thiol- and amino-residues of adjacent nucleoprotamine-fibers.

KEY WORDS: zinc, chromatin decondensation, chromatin stability, human ejaculated spermatozoa, seminal plasma.

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Introduction

The ultimate fertilizing potential of the spermatozoon lies in its nuclear chromatin and the mission of the spermatozoon is not fulfilled until its nucleus has unravelled the chromosomal fibers and made an intact genome available for development of the zygote. Unlike the ovum and somatic cells, spermatozoa lack enzyme systems for repair of the DNA (Matsuda et al. 1985). It is possible that the unique compaction and dehydrated state of the sperm chromatin offers sufficient protection to the genome during its transfer to the ovum, thereby compensating for the lack of repair systems. Condensation of the sperm chromatin occurs in the testis, where the histones of somatic type are exchanged for protamines (Courtens 1983). In eutherian mammals, these protamines are rich in cysteine-residues, i.e., thiol-groups (-SH) which, in some way, contribute to the extremely high resistance of the nucleus to exogenous chemical and physical strains in vitro and the reduced affinity for DNA-affecting drugs and dyes (Miescher 1878, Gledhill 1971, Zirkin et al 1985). However, in vitro studies have shown that mature spermatozoa, treated with thiol-containing compounds like dithiothreitol (DTT), react with a rapid decondensation when exposed to the detergent sodium dodecyl sulphate (SDS) (cf Zirkin et al. 1985). Furthermore, in spermatozoa treated with DTT, the DNA again becomes available for DNA-affecting drugs like actinomycin D (Gledhill 1971) and proteolytic enzymes (Kolk and Samuel 1975, Marushige and Marushige 1978). Thus, it seems as if the forces, which could be changed by the action of compounds containing thiols, stabilize the chromatin and protect it during the transfer to the ovum. What is then the nature of these forces? They are broken by thiols and they resist the repulsion, that SDS could induce between the nucleoprotein fibres (Kvist 1980d). SDS demembranizes the sperm nucleus and penetrates into the nuclear matrix (Kvist et al. 1985), but the naked nucleus still preserves its quaternary structure (Fig 1). Based on the assumption that the only relevant action

This paper was presented at the Symposium on 'Cell Structure and Cell Biology' in honor of Björn Afzelius, December 19 and 20, 1985 in Stockholm, Sweden.

of thiols like DTT is cleavage of disulfide-bridges, most authors conclude that the structural stability of the sperm chromatin depends on disulfide crosslinks (Bril-Petersen and Westenbrink 1963, Calvin and Bedford 1971, Bedford et al. 1973b, Zirkin et al. 1985). However, the observation that ejaculated spermatozoa can decondense in SDS if exposed to a zinc-chelating medium, i.e., in the absence of exogenous thiols, indicated that nuclear zinc contributes to the stability of the sperm chromatin (Björndahl and Kvist 1985, Björndahl 1986) (Fig 2). Moreover, the idea that thiol-groups in the chromatin oxidize into disulfide-bridges during sperm maturation (Calvin and Bedford 1971), is mainly based on the observation that the amount of detectable thiol-groups in mature spermatozoa is low and that the thiol-groups which have been 'lost' during maturation can be recovered with DTT-treatment. However, disappearance of thiol-groups does not necessarily mean that they have been committed into disulfide-bridges, since thiols could 'escape detection', e.g., through formation of stable complexes with divalent cations like Zn^{2+}. Furthermore, besides being a disulfide-bridge cleaving agent, DTT is a very efficient zinc-chelating agent, which selectively releases zinc from human spermatozoa (Kvist and Eliasson 1978). Thiols like DTT could thus destabilize the nucleus and 'recover' thiol-groups in the chromatin by breaking zinc-mediated inter-molecular bindings between thiol-groups in cysteine molecules of the protamins. Others have shown that mature spermatozoa do contain zinc, and that most of the zinc is associated with sulfur-rich structures. Moreover, release of zinc, induced by ethylene diamin (EDTA), is facilitated by agents interacting with single thiol-groups, e.g., iodoacetamide (Calvin et al. 1973, Calvin and Bleau 1974, Calvin et al. 1975).

Chromatin stability and zinc

One way to obtain information about the forces that stabilize the chromatin is to study chromatin decondensation. The process of decondensation has been extensively studied in vitro with various detergents, e.g., SDS. Some infertile men have large proportions of spermatozoa which decondense in the absence of thiol-containing compounds, i.e., in SDS alone (Bedford et al. 1973a, Kvist and Eliasson 1980). This decondensation starts in the posterior region of the condensed chromatin, and continues anteriorly until the chromatin is dissolved and practically undetectable in phase-contrast microscopy (Kvist et al. 1980). This pattern of decondensation corresponds to that observed in the ooplasm (Bedford 1972, Soupart 1976). We found that these infertile men had a relatively low contribution to the ejaculate of the zinc-rich prostatic fluid. In addition, we found that immediate dilution of the ejaculate at ejaculation increased the proportion spermatozoa decondensing in SDS (Kvist 1980a). These results gave indirect evidence for the idea that some factor of prostatic origin helps to stabilize the chromatin of mature spermatozoa.

We also found that normal seminal plasma and seminal plasma dominated by prostatic fluid (obtained from the first fractions of split-ejaculates or from men lacking seminal vesicles) prevented decondensation (Kvist 1980b, Aryer et al. 1987). Furthermore, Zn^{2+}, but not Ca^{2+} and Mg^{2+} (which are also normally present at high concentrations in the seminal plasma), had the same stabilizing effect as prostatic fluid (Kvist 1980c). In addition, samples with spermatozoa which were stable in SDS alone had a high proportion spermatozoa decondensing in SDS if they were also treated with EDTA or albumin - both known to release zinc from spermatozoa (Kvist 1980d, Roomans et al. 1982). These observations strongly support the idea that a divalent cation of prostatic origin acts as reversible stabilizer of the mature sperm chromatin, and that this cation is Zn^{2+}.

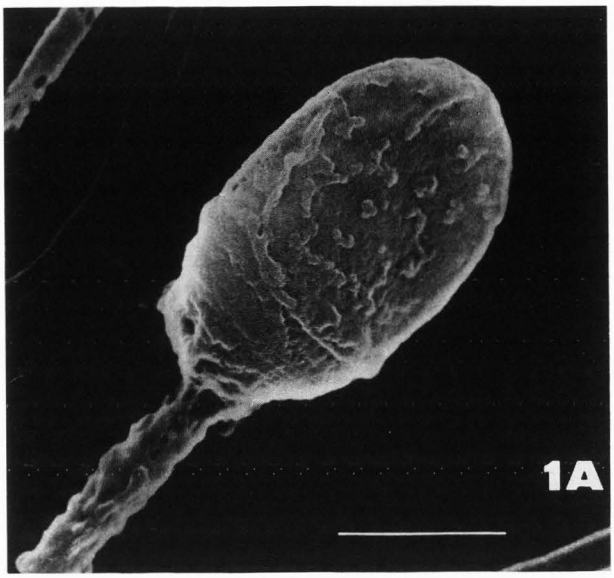
The initial stability of the chromatin was found to be superseded by another stability rather soon after ejaculation. Within five minutes after ejaculation 90% of the spermatozoa could decondense in SDS with EDTA, but after the first hour half of the spermatozoa had become resistant to decondensation in SDS-EDTA (Björndahl and Kvist 1985). Furthermore, we found that the development of this second type of stability in fact was enhanced among spermatozoa which had been depleted of zinc and retarded among spermatozoa which had been supplemented with zinc (Kvist and Björndahl 1985). However, spermatozoa with this 'second type' of 'EDTA-resistant' stability, still could decondense if they were exposed to an excess of thiols, e.g., DTT or cysteine (Kvist and Eliasson 1978).

From the results of these studies on chromatin decondensation we inferred (1) that Zn^{2+} contributes to the stability of the sperm chromatin; (2) that a second type of stability, which probably is due to formation of disulfide-bridges in vitro, supersedes the zinc-dependent stability in vitro; (3) that Zn^{2+} prevents the development of the second type of stability; and (4) that both effects of zinc could be explained by Zn^{2+} interacting between thiol-groups in the chromatin.

Sperm chromatin zinc

Direct evidence that a high content of nuclear zinc is secured at ejaculation was provided in studies where the elemental composition of individual sperm heads was studied with X-ray microanalysis. Other authors have measured the contents of zinc in washed pellets of whole spermatozoa from different regions of the male reproductive tract and reported an accumulation of zinc before (rat: Saito et al. 1967) or upon ejaculation (human: Janick et al. 1971; dog: Saito et al. 1967). With X-ray microanalysis of individual sperm heads we could verify that the nuclei of ejaculated human spermatozoa contain significant amounts of zinc, and that some 90% of this zinc can be released by treatment with EDTA soon after ejaculation (Roomans et al. 1982).

Furthermore, we found significantly lower concentrations of zinc in heads of human spermatozoa taken from the epididymis or vas deferens than in the heads of ejaculated spermatozoa (Kvist et al. 1985, Björndahl et al. 1986). We therefore concluded that in man the level of zinc in the sperm nucleus is regulated at ejaculation.



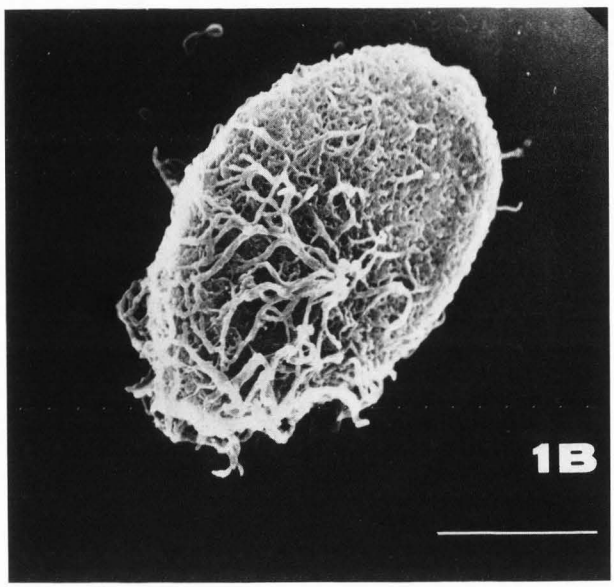


Fig 1A: Appearance of an intact human sperm head in scanning electron microscopy (photo: Lennart Nilsson, Karolinska Institute). Bar = 2 μm.

Fig 1B: Human sperm head exposed to SDS: membrane and nuclear envelope has disappeared and the nuclear chromatin remains stable, i.e., it has not decondensed (photo: Lennart Nilsson, Karolinska Institute). Bar = 2 μm.

Chromatin stability, zinc, and fertility

The amount of zinc in the sperm nucleus and the stability of the chromatin in SDS was studied in fertile men and in men living in barren unions.

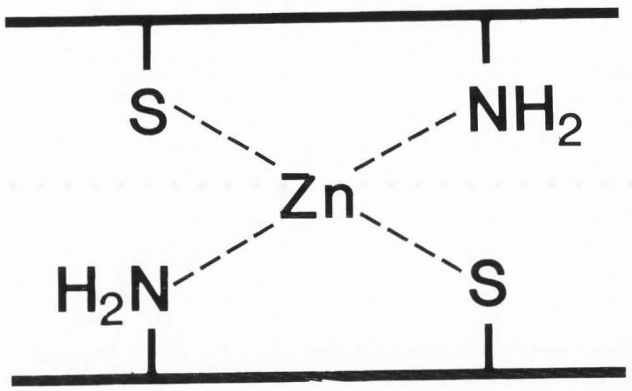


Fig 2: A hypothetical view of how zinc can stabilize the nucleoprotein fibres in the chromatin by interaction with thiol-groups (-SH) of cysteine and amino-groups ($-NH_2$) of arginine in sperm protamines.

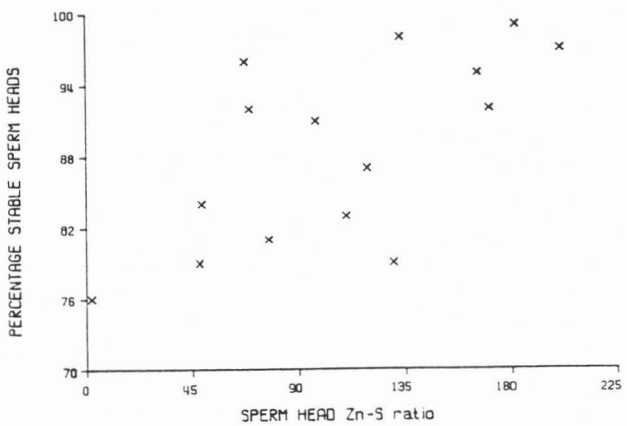


Fig 3: Distribution of nuclear zinc content (1000 * Zn/S intensity ratio) and chromatin stability (% stable spermatozoa in SDS) among men in infertile unions and five fertile donors.

Materials and Methods

Spermatozoa were obtained from five men participating as semen donors in an insemination program, and from 10 men living in unions with hitherto unexplained infertility (all patients and their partners had normal results of routine investigations for causes of infertility, such as sperm count, motility, ovulation cycles, and cervical mucus test. Decondensation of sperm chromatin was studied in 1% SDS in 0.05 borate buffer (pH 9.0) (as previously described: Kvist 1980d, Björndahl 1986). Percentage unreacted (stable) sperm heads was assessed in phase-contrast microscopy after 60 minutes exposure to SDS at 22°C. Zinc content in sperm heads was determined with X-ray microanalysis and expressed as the intensity ratio between zinc and sulfur (1000 x Zn/S), where sulfur was used as an internal standard for the amount of biological specimen examined (Roomans et al. 1982, Kvist et

al. 1985, Björndahl et al. 1986). Statistical analysis was performed with the Mann-Whitney U-test and the Spearman Rank Correlation test (Siegel 1956).

Results

The results were: (a) fertile men had higher proportions spermatozoa which were stable in SDS alone, than had the infertile men (Table 1); (b) sperm nuclei from fertile men had a higher content of zinc than those from the infertile men (Table 1); (c) there was a significant, positive correlation between the content of zinc in the sperm chromatin and the proportion spermatozoa stable in SDS alone (r_s=0.51; p<0.05; N=15)(Fig. 3).

Conclusions

The above reviewed results have led us to the following conclusions:
(1) That Zn^{2+} contributes to the stability of the quaternary structure of the sperm chromatin;
(2) That a second type of chromatin stability, involving disulfide-bridge formation, can develop in vitro;
(3) That Zn^{2+} can prevent the chromatin from becoming superstabilized by this second type of stability;
(4) That, in man, a specific role of the prostate is to secure a high content of zinc in the sperm nucleus (5) That subnormal content of zinc jeopardizes the normal stability of the chromatin and could cause, or at least signify, a low fertilizing potential of the spermatozoon.

It seems justified also to suggest that release of zinc from the sperm nucleus in the ovum would facilitate a physiological decondensation.

The mode of action of Zn^{2+} is not known. One tentative explanation, in congruence with our results, is that Zn^{2+} interacts with thiol-groups of cysteine and e.g., amino-groups of arginine in the protamines (Fig 2).

Acknowledgements

Dedicated to Björn A. Afzelius, a good friend and a brilliant and inspiring scientist, who is always eager to learn.

Financial support was obtained from the Swedish Medical Research Council (project 04X-07153), from the Swedish Work Environment Funds (project 85-0364), and from the Karolinska Institute.

Table 1

Comparison of sperm chromatin stability after 1 h exposure to SDS (1% Sodium Dodecylsulfate) and sperm chromatin content of zinc (X-ray microanalysis) between fertile donors and men in infertile marriages. Significances for differences between groups calculated with the Mann-Whitney U-test (Siegel 1956).

	% stable sperm heads (mean, range)	Sperm head content of zinc (Zn/S) (mean, range)
Fertile donors (N=5)	95 (91-99)	166 (97-182)
Men in infertile marriages (N=10)	84 (76-97)	94 (48-201)
significance for difference between groups	p<0.05	p<0.05

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Discussion with Reviewers

B. Afzelius: The first study of the resistance to swelling of mammalian sperm evidently is that by Calvin and Bedford (1971). In that paper they point out that the more immature spermatozoa from the epididymal caput are more stable than the more mature cauda spermatozoa when aged in vitro. Can you give an explanation?

Authors: Apparently, oxidation of thiols into disulfide-bridges is hindered in more mature spermatozoa from cauda epididymis. This could be due to a steric change in the chromatin, or to a binding of other compounds to thiols during the passage through the epididymis, thereby preventing thiol-groups from taking part in further formation of disulfide-crosslinks. From our point of view, incorporation of zinc that interchelates between thiol-groups, could alone explain the high chromatin stability of spermatozoa from cauda epididymis, the low amount of detectable thiol-groups in these sperm heads, and the weak tendency to form further disulfide-crosslinks when aged in vitro.

B. Afzelius: You show here that some cases of male infertility (or decreased fertility) is correlated with a lowered uptake of zinc by the spermatozoa which presumably is the reason of the low fertility. Will this finding lead to some treatment of the patients, such as zinc injections in the blood, increased amounts of zinc in the food, or some such treatment?
Authors: A normal secretory function of the prostate, measured as concentration of zinc in seminal plasma, does not guarantee an appropriate uptake of zinc into the sperm chromatin. It seems that the sequence of ejaculation is of importance since an early admixture of seminal vesicular fluid severely limits the fraction of zinc available for uptake (Björndahl 1986). However, in cases with low secretion of zinc from the prostate, efforts to increase the accumulation of zinc in the prostate may be beneficial. Accumulation of zinc in the prostate seems to be dependent on androgens and it has been suggested that citrate production induced with androgens would be a mechanism responsible for accumulation of zinc (Arver 1982). We have recently initiated a study with the aim to find out whether treatment with androgens during a short period of time could increase the amount of zinc that is secreted from the prostate and available for uptake into the sperm chromatin.

G.M. Roomans: Colonna and Oliphant (1986) recently showed, using X-ray microanalysis, substantially lower Zn levels associated with the principal piece of both capacitated and acrosome-reacted sperm than with ejaculated cells (in the rabbit). Could you comment on these findings in the light of your own theory?
Authors: Colonna and Oliphant compared ejaculated spermatozoa and spermatozoa exposed to their capacitation medium, which has high ionic strength. This treatment induced several changes in elemental composition. Spermatozoa with reacted acrosome and which had been 'capacitated', had higher content of zinc in the principal piece, but lower levels in the acrosome and mid-piece. The significance of this 'redistribution' is not known. In contrast, they found no change in the levels of zinc in the nuclei of ejaculated, 'capacitated' spermatozoa, and spermatozoa with reacted acrosome. However, if we consider that a premature release of zinc may occur before fertilization, this would result in two possible outcomes, dependent on the redox-state: (1) in a reductive environment: a partial, premature decondensation, which makes the genome available for enzymatic degradation; (2) in an oxidative environment: oxidation of unprotected thiols into stabilizing disulfide-crosslinks, which may retard or abolish the eventual decondensation in the ooplasm.

G.M. Roomans: Your findings shown in Table 1 of lower Zn levels and lower stability in spermatozoa of infertile men are very intriguing. However, even the infertile group has 84% stable spermatozoa and both the range of stability and the range of Zn content overlaps that of healthy men. Therefore, I wonder whether in an individual case, the infertility can be explained by a decreased zinc content.
Authors: Infertile couples should make a heterogeneous group with respect to the causes of infertility. The present results open the possibility that a low content of zinc in the sperm chromatin could be one factor causing infertility. However, men in couples with other causes of infertility must be expected to have contents of zinc in sperm nuclei that overlap the values from fertile donors.

L. Plöen: You have shown that zinc is important for chromatin stability. Could you speculate on other factors that might be involved in chromatin decondensation?

Do you know if there are any differences in the mechanism of decondensation in vitro and in vivo (fertilization)?
Authors: The mechanism by which decondensation of ejaculated spermatozoa proceeds in vivo is not known, but this decondensation bears morphological similarities to decondensation induced by SDS with EDTA.

The DNA-protamine complex that is to be decondensated could be regarded as a temporary, physiological aggregate of macromolecules surrounded by a nuclear envelope and the plasma membrane. A prerequisite for decondensation is the disappearance of these membranes. In our in vitro system, this is performed with SDS. In vivo, the plasma membrane fuses with the vitelline membrane during penetration, and there is a rapid break down of the nuclear envelope, most probably caused by factors in the ooplasma.

At least two types of bindings are then to be disrupted in the nuclear aggregate. Weaker forces, possibly hydrogen bonds, can be broken by inclusion of repulsive forces with e.g., detergents like SDS in vitro. In vivo, phosphorylation of protamines and rehydration could be two mechanisms that cause the repulsion of the fibers.

The stronger forces could be salt-bridges, with divalent cations, or they could be covalent bridges. Since a combination of zinc-chelating compounds and SDS can bring about a complete decondensation of mammalian spermatozoa in vitro, it is possible that salt-bridges are present. The evolution of cysteines in the protamines, makes the formation of disulfide-bridges possible. Such bridges may stabilize the secondary structure of the protamine itself, i.e., intra-molecular disulfide-bridges. The thiol groups could also contribute to the stability of the quaternary structure, either in intermolecular disulfide-bridges or through formation of intermolecular salt-bridges with zinc. The latter type of complex would have a strength which is comparable with that of covalent disulfide-bridges.

If salt-bridges with zinc constitute the strong intermolecular forces, then decondensation in vivo could be achieved by removal of zinc concomitant with a repulsion of chromatin fibers.

If also intermolecular disulfide-bridges contribute to the physiological stabilizing forces, these have to be broken. One way is through reductive cleavage by free thiol groups, which can be added in vitro (e.g., DTT or mercaptoethanol), or supplied in vivo: by the ooplasm (reduced glutathione, redoxine) or provided, as free thiol groups, by the protamines

themselves. If thiol-groups in the protamines participate in cleavage of intermolecular disulfide-bridges, then the net effect of decondensation would be an oxidative process (i.e., exchange of intermolecular disulfide-bridges into intramolecular disulfide-bridges), which, from a thermodynamic point of view would be favoured. A main role for free thiols in the ooplasm could then be to initiate this process.

Epidydimal spermatozoa failed to decondense in ova exposed to treatments that block thiol-groups, oxidizes GSH, or blocks the production of GSH (Calvin et al. 1986, Perreault et al. 1984). It is, however, not known to what extent the 'unphysiological' usage of epididymal spermatozoa creates a need for thiols in the ooplasma, since epidydimal spermatozoa are susceptible to disulfide oxidation when handled in vitro. If thiols to higher extent were protected from oxidation by zinc, as is the case with ejaculated human spermatozoa, then the need for thiols in the ooplasm to support decondensation of spermatozoa would be lower.

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