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Semen Analysis

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In view of the well known sensitivity of the seminiferous epithelium to various stress factors and to chemicals one would expect that semen analyses would be part of many screening programs to detect dangerous chemicals and environmental hazards. This is not the case, and our knowledge about the production and functional properties of human spermatozoa is mainly based on analyses of specimens from men with barren marriages. A more common use of semen analyses should enable us to better define the normal limits for many of the potentially relevant variables. Due consideration must, however, also be given the time for spermatogenesis and for transit through the epididymis as well as the influence of seminal plasma factors on many functional properties of the spermatozoa. From the scanty information available one can already now presume that careful analyses of motility and morphology of the spermatozoa under standardized conditions will be of help in the early detection of environmental hazards. A more common use of methods for the assessment of such functional properties of the spermatozoa as structural stability, membrane permeability, metabolism, and resistance to physical stress will give additional information about the effects of chemicals and other factors. To exploit these potential methods for the early detection of environmental hazards there is, however, also a need of a changed attitude towards semen analysis from the medical profession as well as from the public.

In view of the fact that the reproductive organs are part of the organism and are sensitive to many forms of stress, semen analyses can be of interest to those who work with medical aspects of environmental and toxicological problems. Most toxicologists have, however, given this organ system little attention, and the information available concerning the effects of chemicals and environmental factors on the reproductive system is too fragmentary to allow more than a general discussion about some basic aspects.

Our insufficient knowledge is not primarily due to a lack of interest on the part of toxicologists, but to other factors. Moral and prejudiced opinions, for example, have led to a severe bias in the selection of men whose semen has been analyzed. An additional problem is that laboratory data have been expressed in a "diagnostic" terminology. Azoospermia or oligozoospermia, for example, are only symptoms and must not be understood as a "diagnosis." Men are frequently incorrectly classified as "fertile" or "infertile" on the basis of one or more semen characteristics.

Very little attention, in any systematic way, has been given the physiological properties of human spermatozoa and the specimens have rarely been obtained from carefully examined men. The results have mainly been related to sperm density (misleadingly expressed in terms of "fertility") and consequently not to other and probably more important parameters, e.g., the secretory function of the accessory genital glands (*1–3*).

A terminology should be clear and correct, and remove the more common misunderstandings. The terminology used in this presentation is given in Table 1. "Fertile semen samples" refer to specimens obtained from men whose wives had become pregnant within three months before or after the semen was examined and there was no obvious reason to believe that the man could not be the potential father. In most cases the semen sample was obtained after the conception.

Semen analyses of potential usefulness to the toxicologists can be divided into two main groups: (a) the standard analyses, and (b) studies of functional parameters of the spermatozoa.

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Table 1. Terminology related to male fertility.

Facts	Terminology	
	Correct	Incorrect
One or more children	Father	Fertile
No children and not tried	Fertility unknown	
No children but tried > one year	Infertile	Subfertile Sterile
Wife has recently become pregnant	Fertile[a]	
Normal values in semen analysis	Normal semen analysis	Fertile
Subnormal values in semen analysis	Objective descriptions	Subfertile, Infertile, Sterile
No spermatozoa in the ejaculate	Azoospermia	Sterile[b]

[a] Valid unless there are objective data proving that the man cannot be responsible for the pregnancy.

[b] "Sterile" is a diagnosis and should be used only when the laboratory and clinical examinations have proven the man to be sterile. Azoospermia is a symptom.

Standard Analyses

The standard analyses include semen volume, viscosity, liquefaction, and number, motility and morphology of the spermatozoa. A change in the volume will most likely reflect an altered secretory function of the prostate or the seminal vesicles, and biochemical analyses of the seminal plasma will then be of importance. Acid phosphatase, citric acid, zinc, and magnesium are specific secretory products from the prostate. Most of the liquefying enzymes also originate from the prostate. Fructose and prostaglandins are secreted from the seminal vesicles (*1*).

Sperm density is the most common parameter in studies on the possible effects of drugs or chemical compounds on the male reproductive system. It is important to know the variations in sperm density between several specimens from the same man before an evaluation can be made. Semen samples should preferably be collected over a period of at least three months before the man is exposed to a drug or chemical. A decreased sperm density can be due to a direct effect on the germinal epithelium or and indirect effect due to a suppression of the gonadotropin secretion from the pituitary. Oral administration of diphenylhydantoin (Dilantin) can cause an oligozoospermia due to the second mode of action (*4*). However, an infection with fever will also cause a severe depression of the sperm production (*1*).

In the assessment of sperm density it is important to recall that a low sperm count (e.g., 5 million/ml) is in itself not a reason for infertility (*1*, *5*).

Sperm motility is a standard analysis but also a functional parameter. To be meaningful the motility assessment must include three factors: the mean progressive motility, the per cent motile spermatozoa, and the time after ejaculation. The mean progressive motility is frequently expressed as a score, i.e., 0 = no progressive motility, 1 = poor, 2 = medium, 3 = good, and 4 = very good mean progressive motility. The importance of the time factor can be illustrated by the fact that infections in the prostate frequently cause a rapidly declining motility of the spermatozoa. Such a decline of the motility can be caused by bacterial toxins (*6*) or by deficiencies in the seminal plasma (*3*).

Among 100 semen samples from 50 men whose wives were pregnant in the first trimester we noted that two had a mean progressive motility score assessed as "medium" and the remaining as "good." None had less than 40% motile spermatozoa 30 to 40 min after ejaculation. Four hours later some samples had spermatozoa without progressive motility and a low percentage of motile cells ($\leq$20%). Rapidly declining motility therefore seems compatible with fertility, but a low motility soon after ejaculation can be regarded a sign of decreased fertility. The effects of drugs on the motility of human spermatozoa were recently reviewed (*2*).

Sperm morphology has long been regarded a parameter closely related to fertility. Despite this there is no commonly accepted method for the assessment. Some staining methods do not even allow an evaluation of the whole spermatozoon. It should, however, be a minimal requirement that the whole cell is assessed and not only the head, since semen samples from fertile men extremely seldom have more than 20% spermatozoa with coiled tails while about 15% of the samples from infertile men have more than 20% coiled tails (Eliasson, to be published).

It is possible that coiled tails can be the result of a disturbed function in the epididymis since local heating of the scrotum of rams caused a drastic increase in the percentage spermatozoa with coiled tails two weeks after the heat was applied (*7*). Studies on bull spermatozoa have also given evidence that factors in the epididymis are involved in the development of coiled tails (*8*). A close relation between the zinc content of washed spermatozoa and the percentage of coiled tails was described by Lindholmer (*9*).

In one patient who was frequently exposed to organic solvents I noted significant variations in the percentage of coiled tails. In some specimens it was in the order of 10 to 15%, in others up to 51%. There seemed to be a relation between exposure to organic solvents (glues, paints, and cleaning fluids)

and the high incidence of coiled tails. Further studies are in progress.

Exposure to lead can cause a significant increase in the percentage of spermatozoa with morphological defects, as illustrated in Figure 1 (*10*). The abnormal spermatozoa included binucleated, amorphous, and tapered forms. The lead-exposed men also had a higher frequency of semen samples with low sperm concentration and poor sperm motility than the controls. Morphological changes induced by chemicals and stress have also been reported by others (*11–13*).

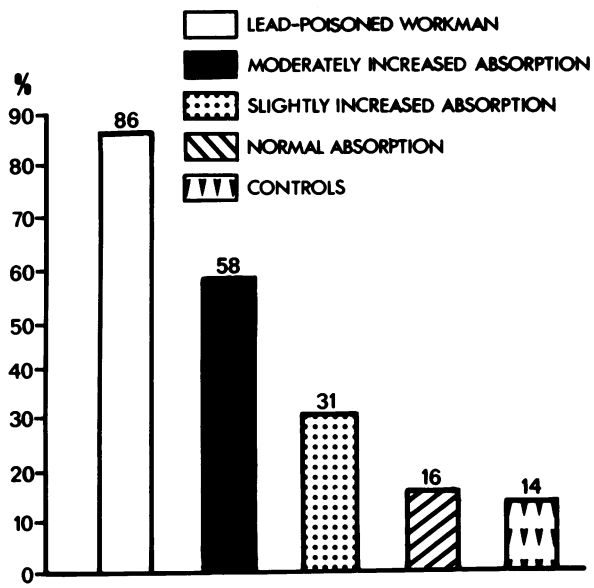

FIGURE 1. Incidence of abnormal spermatozoa in semen samples from workmen with long-term lead exposure and controls (*10*).

Functional Parameters

Some functional parameters that could be useful in studies on the possible effects of chemicals and environmental factors on the male reproductive system and male fertility are motility and survival in seminal plasma, motility in cervical mucus, resistance to physical stress, metabolism, resistance to sodium dodecyl sulfate (SDS), uptake of zinc, and release of enzymes, proteins, and lipids. Motility in the seminal plasma is (as discussed above) normally assessed by a subjective rating. It is therefore important to use a supravital staining technique (e.g., with eosin Y) as a complement. The stain does not penetrate into a live cell and has in several studies proved useful for differentiation of live from dead spermatozoa (*14*). In most semen samples the sum of the percentage of motile and dead spermatozoa, respectively, is close to 100. A complete lack of motility does not necessarily mean that all spermatozoa are dead (*15*).

The relation between sperm penetration into cervical mucus and migration in this medium and fertility has been investigated by several authors (*16–18*). On the other hand, nothing seems to have been published about the possible effects of drugs on this parameter.

The resistance of human spermatozoa to stress factors like gravitation force (centrifugation), dilution, and low temperature can be decreased by prostatic dysfunctions (*19*) and probably also by several other factors (*20*). There has been no systematic study of the possible relation between the resistance of the spermatozoa to physical stress and fertility in man, nor of the effects of drugs or chemicals.

Application of heat (40°C) for 3 hr to a ram scrotum results in severe changes in the metabolism and morphology of the spermatozoa (*7*). Some of the results are presented in Table 2. The changes in the spermatozoa became evident within two weeks and must therefore have been induced while the cells were in the epididymis or during the very late phase of spermatogenesis.

Table 2. Effect of local heat (40°C) applied for 3 hr to the scrotum of rams on metabolism and structure of the spermatozoa.[a]

Time	Observation
Day 1	Decreased oxygen consumption; no other changes
Week 1-2	Decreased motility, glycerol breakdown, and lactic acid production
Week 2-3	Damaged plasma membranes Increased percentage of coiled tails and loose heads
Week 9-10	Morhology and metabolism have return to normal

[a] Modified from Brown-Woodman et al. (*7*).

Ejaculated human spermatozoa are normally resistant to exposure to a 1% solution of sodium dodecyl sulfate (SDS) for 1 hr. Men with infertility problems have a higher percentage of semen samples with a decreased sperm resistance to SDS than healthy volunteers (*21*). Semen samples with biochemical changes indicating a prostatic dysfunction frequently have a high percentage of spermatozoa that are affected by SDS. Addition of zinc to such specimens can significantly increase the resistance as is illustrated in Figure 2. Magnesium and calcium have no such effect (*22*). The spermatozoa develop resistance to SDS during the passage through the epididymis, and it is therefore possible that the resistance to SDS can be an indirect measure of the maturation that spermatozoa undergo in this organ.

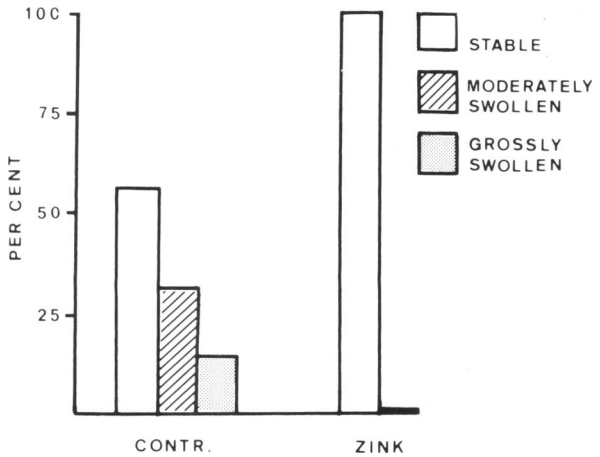

FIGURE 2. Incidence of stable, moderately swollen, and grossly swollen human spermatozoa incubated for 1 hr in 1% sodium dodecyl sulfate (SDS) in a buffered salt solution with and without 1.6 mmole zinc/l. (*22*).

Spermatozoa from healthy volunteers and from men whose wives are pregnant in the first trimester have a remarkably constant zinc content (2 to 4 $\mu g/10^8$ spermatozoa). Spermatozoa from many men with infertility problems (independent of sperm concentration) and/or biochemical signs in the seminal plasma of a prostatic dysfunction, on the other hand, frequently have a zinc content of the order of 20 to 100 $\mu g/10^8$ spermatozoa (or higher). There is indirect evidence that zinc uptake after ejaculation could be a functional parameter related to viability, motility, and fertility of human spermatozoa (*3*).

Release of enzymes, specific proteins, and phospholipids by bull, ram, and boar spermatozoa have been regarded functional parameters related to fertility (*23, 24*). There is a need for corresponding studies on human spermatozoa.

The relevance of physiological and biochemical research in relation to semen properties and fertility was recently reviewed by Mann (*25*).

Concluding Remarks

Semen analyses could be very useful in studies on the possible effects of drugs, chemicals, and environmental hazards on testicular function, epididymal function, and on male fertility. They will also be useful in studies aiming at a better understanding of the excretion of drugs into the seminal plasma and the effects of drugs, organic solvents, and other chemical hazards on functional properties of the spermatozoa. We have some techniques available for such studies. However, more and better techniques as well as a better understanding of the physiology of the accessory genital glands and the spermatozoa under normal conditions are prerequisites to make full use of semen analyses in the evaluation of possible hazards related to chemicals and environmental factors.

The most effective way to learn if environmental factors will influence the semen properties would be to analyze semen samples (at least two) before a given man will be exposed to the potentially harmful chemical(s) and then to follow him with semen analyses at least every third month. In addition to the routine analyses, one should include several of the functional parameters and as well as possible document the potential fertility of the man. The fertility must not be assessed from the semen analyses nor should the laboratory reports concerning the properties of the semen samples contain any "diagnostic" terminology. It would probably be most valuable to make corresponding studies also on men who will leave a job which could have involved potential hazards to their health and to follow them for many months after they have left such a job. The successful accomplishment of studies along these lines will, however, require a change in the general attitude to semen analyses, particularly from members of the medical profession.

REFERENCES

1. Eliasson, R. Analysis of semen. In: Progress in Infertility, Vol. II. S. J. Behrman and R. W. Kistner, Eds., Little, Brown and Co., Boston, 1975, pp. 691–713.
2. Eliasson, R. Pharmacological actions on the production and physiology of mammalian spermatozoa. In: Sperm Action (Progr. Reprod. Biol. Vol. 1), P. O. Hubinont and M. L. Hermite Eds., Karger, Basel, 1976, pp. 284–293.
3. Eliasson, R., and Lindholmer, C. Functions of male accessory genital organs. In: The Human Semen and Fertility Regulation in Men. E. Hafez., Ed., Mosby, St. Louis, 1976, pp 44–50.
4. Stewart-Bentley, M., et al. Effects of dilantin on FSH and spermatogenesis. Clin. Res. 24: 101A (1976).
5. van Zyl, J. A., et al. Oligozoospermia: a seven-year survey of the incidence, chromosomal aberrations, treatment and pregnancy rate. Int. J. Fertil. 20: 129 (1975).
6. Teague, N. S., Boyarski, S., and Glenn, J. F. Interference of human spermatozoa motility by Escherichia coli. Fertil. Steril. 22: 281 (1971).
7. Brown-Woodman, P. D. C., et al. Metabolic and ultrastructural changes in ejaculated spermatozoa induced by heating the testes of rams. J. Reprod. Fert. 46: 501 (1976).
8. Swanson, E. W., and Boyd, L. J. Factors affecting coiled-tail spermatozoa in the bull. Amer. J. Vet. Res. 23: 300 (1962).
9. Lindholmer, C. Toxicity of zinc ions to human spermatozoa and the influence of albumin. Andrologia 6: 7 (1974).
10. Lancranjan, I., et al. Reproductive ability of workmen occupationally exposed to lead. Arch. Environ. Health 30: 396 (1975).
11. Ulstein, M., et al. Changes in sperm morphology in normal men treated with Danazol and testosterone. Contraception 12: 437 (1975).

12. Wyrobek, A. J., and Bruce, W. R. Chemical induction of sperm abnormalities in mice. Proc. Nat. Acad. Sci. (U.S.) 72: 4425 (1975).
13. MacLeod, J. The semen quality in relation to male infertility. In: Fertility Disturbances in Men and Women. A. Joel, Ed., Karger, Basel, 1971, pp. 127–134.
14. Eliasson, R. Supravital staining of human spermatozoa. Fertil. Steril. in press.
15. Eliasson, R., et al. The immotile cilia syndrome: a congenital ciliary abnormality as an etiological factor in chronic airway infections and male sterility. N. Engl. J. Med. 297: 1 (1977).
16. Kremer, J. The *in vitro* spermatozoal penetration test in fertility investigations. Doctoral dissertion. University of Groeningen. The Netherlands. 1968; van Denderer, Groeningen. 1968.
17. Ulstein, M. Fertility of donors at heterologous insemination. Acta Obstet. Gynecol. Scand. 52: 97 (1973).
18. Davajan, V., and Nakamara, R. M. The cervical factor. In: Progress in Infertility, Vol. II. S. J. Behrman and R. W. Kistner, Eds., Little, Brown and Co., Boston, 1975, pp. 17–46.
19. Hofmann, N. Fertilitätsstörungen und chemische Entzündungen im Genitalbereich (Fortschr. der Andrologie, Vol. 4). Grosse, Berlin, 1975.
20. Smith, K. D., Stultz, D. R., and Steinberger, E. Effects of long-term storage of human spermatozoa in liquid nitrogen. In: Aging Gametes. International Symposium. Seattle, 1973. R. J. Blandau, Ed., Karger, Basel, 1975, pp. 265–277.
21. Bedford, J. M., Bent, M. J., and Calvin, H. Variations in the structural character and stability of the nuclear chromatin in morphologically normal human spermatozoa. J. Reprod. Fert. 33: 19 (1973).
22. Kvist, U., and Eliasson, R. Zinc dependent chromatin stability in human ejaculated spermatozoa. Paper presented at 1st International Congress of Andrology, Barcelona, July 12-15, 1976; Int. J. Andrology (Suppl. 1), In press.
23. Larsson, K., and Einarsson, S. Fertility and postthawing characteristics of deep frozen boar spermatozoa. Andrologia 7: 25 (1975).
24. Darin-Bennett, A., Poulos, A., and White, I. G.The effect of cold shock and freeze-thawing on release of phospholipids by ram, bull, and boar spermatozoa. Austral. J. Biol. Sci. 26: 1409 (1973).
25. Mann, T. Relevance of physiological and biochemical research to problems in animal fertility. Proc. Roy. Soc. (London) B193: 1 (1976).