Sperm DNA: organization, protection and vulnerability –
from basic science to clinical application

Changes in human sperm chromatin – from testis to ejaculate and beyond

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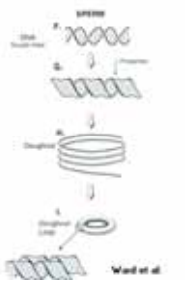
Miescher 1878

The chromatin rope.

- The ” rope” of sperm chromatin is composed by three strings
- The two DNA-strands and the third is the string of protamine-monomers.



The sperm nucleus closes!

- These changes in the DNP express themselves as
- a decrease in stainability by the Feulgen reaction
- a lowered capacity to bind basic dyes
- a decrease in the ability of the complex to bind H*-Actinomycin D.
- Inactivation of the genome + condensation of the chromatin

Nils Ringertz, Barton Gledhill Zebigniew Darynkiewicz Expt Cell Res 1970

Chromatin condensation

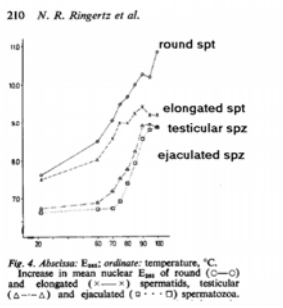


Fig. 4. Abscissa: E_{mn}; *ordinate:* temperature, °C. Increase in mean nuclear E_{mn} of round (○—○) and elongated (×—×) spermatids, testicular (△—△) and ejaculated (□ · · · □) spermatozoa.

In salmon, the protamins consist mainly of Arginine residues:

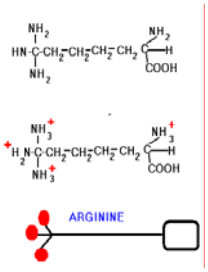
CONCLUSION?

Enough to make possible chromatin condensation

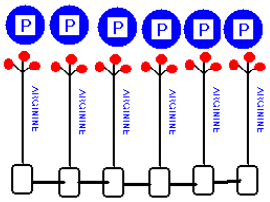
Arginine is the most common amino-acid in protamines

ARG is a basic amino acid having extra – NH_2 NH2 residues which can accept free hydrogen H^+

the positively charged NH_3^+ groups can act to neutralize the negatively charged phosphate-groups of the the DNA-back-bone.

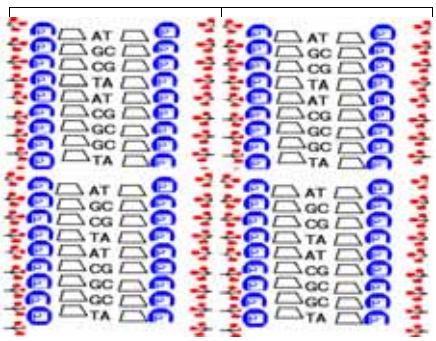


Arginine NH_3^+ neutralizes negative charges on DNA Phosphate groups



•Balhorn calculated that the arginines in one protamin monomer neutralizes the 20 phosphate groups belonging to one turn of the DNA helix, i.e. 10 bp.

Allow packaging, side by side



Protamines in eutherian mammals

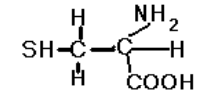
- +Serine +Threonine
- Consequence? can be phosphorylated!

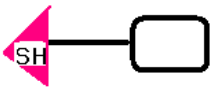
+ phosphates-groups give neg repulsive forces – decondensation in oocyte?

- phosphates-groups allow compaction

- + Histidine offers zinc-binding imino group
- +Cysteine offers reactive S^- .

Human protamines contain 5 (P1) and 6 (P2) Cysteine residues



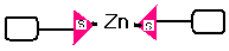


CYSTEINE

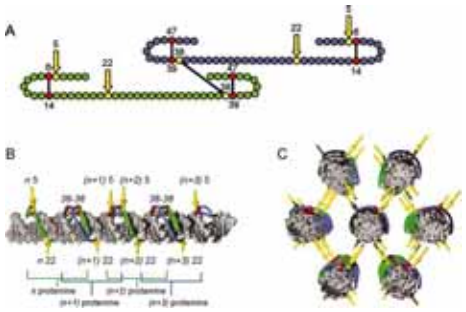
Thiols can interact in

Disulfide-bridge

Salt-bridge
Zinc-bridge

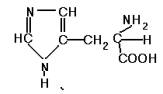


Thiols have high affinity for Zn^{2+}

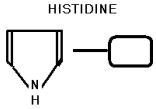


- CYSTEINE in proteins contributes normally to the secundary structure by forming **intra-** molecular disulfide-bridges.
- A role for zinc as a general and temporary stabilzer of various sperm structures seems plausible. Such a role for zinc is not unique Stabilization with zinc is one of the earliest evolved solutions for to stabilize aggregates of macromolecules (Chester 1978).

Protamines contain Histidine. P2 has most histidines



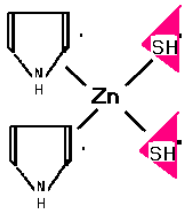
HISTIDINE



The imino-group (-NH) of HIS has high affinity for Zinc

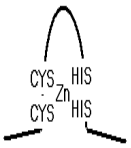
Zinc crossbinding elements of cysteine or/and histidine

Sperm chromatin



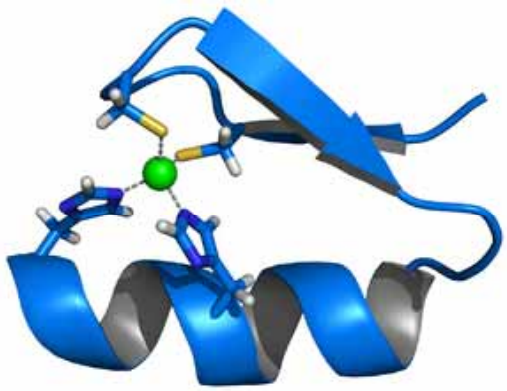
Kjellberg 1993

Other DNA binding proteins



Zinc-finger

Klug & Rhodes 1987



During the transfer from the testis to the cauda epididymis more changes occur in the sperm chromatin.

(1)The resistance to decondense in a detergent (e.g Sodium dodecyl sulphate , SDS) increases and

(2) the resistance to decondense in SDS with e.g. Beta mercaptoethanol or Dithiotreitol (DTT) increases.

(3) The amount of detectable free thiol groups decrease (iodacetamid*)
(4) Disappeared thiols can be re-found after sperm exposure to DTT.

Dominating Conclusions

- (1) Resistance to SDS S-S
- (2) Resistance to SDS+DTT S-S
- (3) Loss of SH S-S
- (4) SH reappears after DTT treatment SH SH

Alternative conclusions including results on zinc

- (1) Resistance to SDS S-Zn-S
- (2) Resistance to SDS+DTT S-Zn-S
- (3) Loss of SH S-Zn-S
- (4) SH reappears after DTT treatment SH SH

How to explain the results?

A) Free thiols disapperar while becoming occupied in forming disulfide-bridges that can be re-opened by DTT.

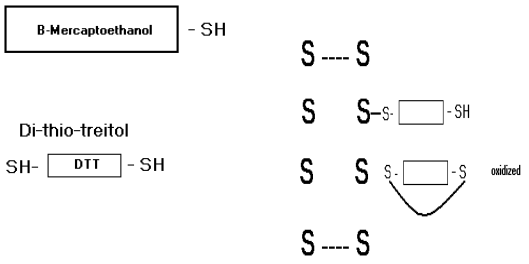
-SH HS- S—S -SH HS-

B) Free thiols disapperar while becoming occupied in forming salt-bridges with Zinc that can be reopened by DTT.

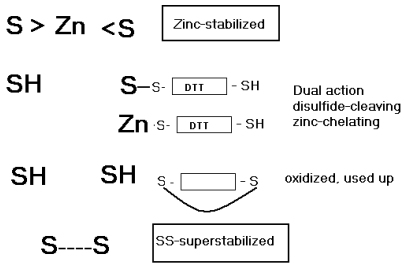
-SH HS- S > Zn < S -SH HS-

C) Both

How thiols open disulfide-bridges!

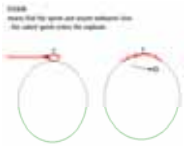


How thiols open zinc-thiol-bridges!



Undressing the sperm DNA

- The plasma membrane was left upon fusion with the oocyte
- The nuclear membranes are thereafter rapidly broken down.
- Left is at least two types of **inter**-molecular bonds
- **Weaker forces** – hydrogen-bonds – can be broken by repulsive forces: phosphorylation, heparans, rehydration; in vitro detergents
- **Stronger** bonds: covalent bonds (S-S) and/or salt-bridges



Physiological ways to introduce repulsive forces in the chromatin.

- Heparin (heparans in the oocyte??)
- Phosphorylation
- For every turn of the DNA there are 10 bp with totaly 20 negatively charged repulsive Phosphate-groups.
- Protamines contain 5,6 or 7 Serine or Threonine residues. that are phosphorylated in the testis. Phosphorylation introduce repulsive forces.

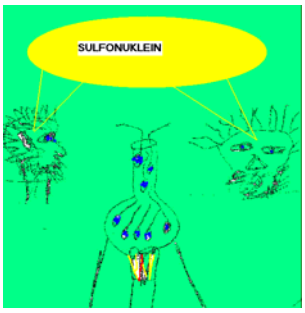
In the oocyte

- Chromatin decondensation could be induced by zinc-removal from the chromatin.
- Thioredoxin or reduced gluthathione could be the zinc-chelators.
- Released thiols may help opening disulfide bridges.

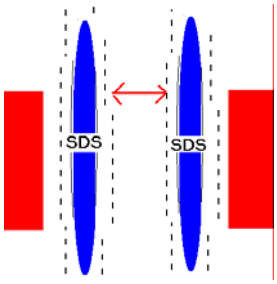
The Sperm, Conceptually…

- The sperm and the DNA-protamine complex are temporary physiological aggregates of macromolecules
- built to resist external influences and
- built to immediately decondense and deliver the DNA and other messages upon arrival in the ooplasm.

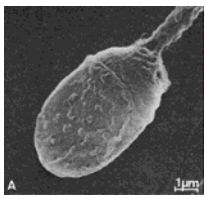
Miescher 1878

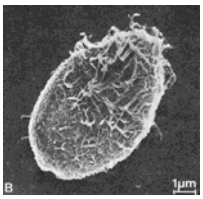


Sodium Dodecyl Sulphate introduces negative repulsive forces



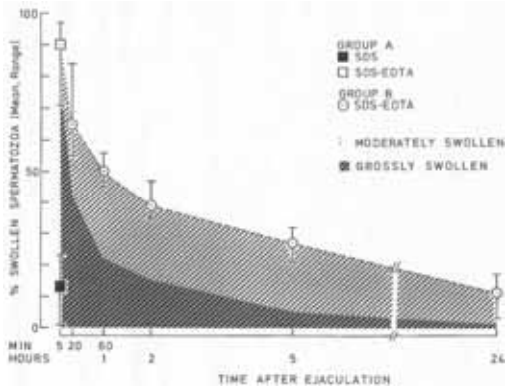
+ SDS





Totally Resistance vs Fast delivery of DNA

- Sulfonuklein
- 90% decondensed < 5min after ejaculation
  If exposed also to zinc-chelating EDTA



But soon…

- At 60 min after ejaculation 50% of the human spermatozoa became resistant to decondesation in SDS-EDTA.
- What does it mean?
- A new type of stability (resistant to decondensation in SDS-EDTA) develops rapidly in vitro.
- Pre-exposure to zinc-chelators like EDTA, Albumine or Cysteine enhanced the development of this type of stability.
- Incubation in zinc containing solution retarded the development.
- Decondensation occurred if exposed to SDS with thiol-compounds like DTT or Cysteine in combination with detergent

And in other mammals

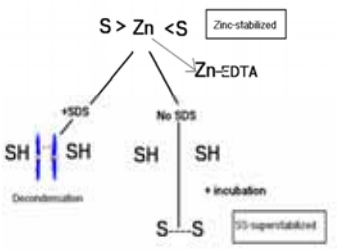
- Decondensation of epididymal and ejaculated spermatozoa from the boar and epididymal spermatozoa from the rat and the hamster can also be facilitated with EDTA (Kvist et al 1987).
- Boar spermatozoa preexposed to cysteine develops a non zinc dependent stabilization (Björndahl et al 1989)

Explanation?

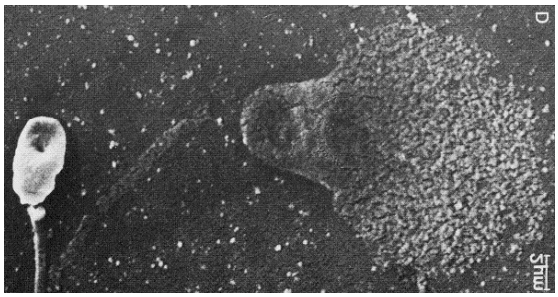
- At ejaculation spermatozoa have a zinc-dependent chromatin stability.
- After ejaculation, the inherent zinc-dependent stability is superseded by a zinc-independent stability involving oxidation of free thiols into disulphide bridges (Björndahl, 1985, Kvist, 1985).
- Oxidative disulfid-bridge formation
- Presence of zinc hinders
- Depletion of chromatin zinc enhances.

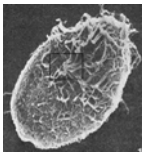
Dual actions by Zinc:(1) stabilizes the structure and (2) prevents oxidation

- Removal of zinc gives two possibilities!
- 1) immediate decondensation
- 2) otherwise develops superstabilization in air atmosphere.



Exposed to SDS-EDTA one resistant, one decondensed

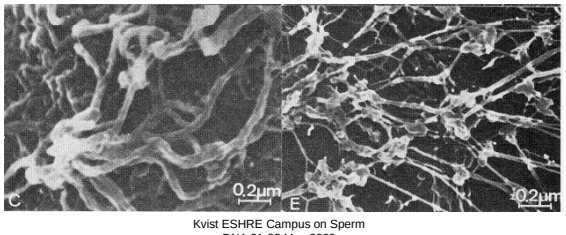


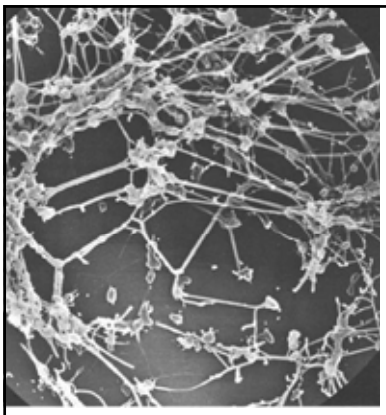


Scanning Electron Microscopy

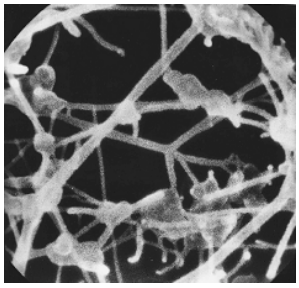
Kvist & Nilsson1980

Exposed to SDS alone (upper left and lower left) and SDS after pre-exposure to EDTA.

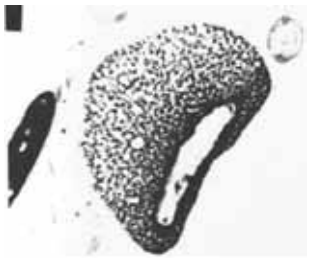




SDS exposed after EDTA pre-exposed at ejaculation



Transmission electron microscopy SDS-EDTA-exposed spermatozoa



1 zinc/1 protamine/ 10 bp DNA

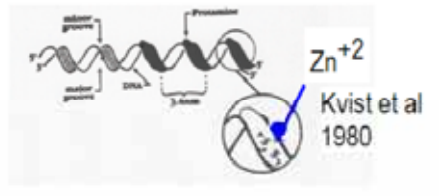
	Zinc/Sulfur x 1000
Fertile men	150 (97-182)
Childless men without prostatic affection	134 (110-201)
Childless men with prostatic affection	62 (48-77)

	Cys	HIS	P SER/THR
Protamin 1	6	1	5
Protamin 2a	5	8	6
Protamin 2b	5	9	7
	5-6	1-9	5-7

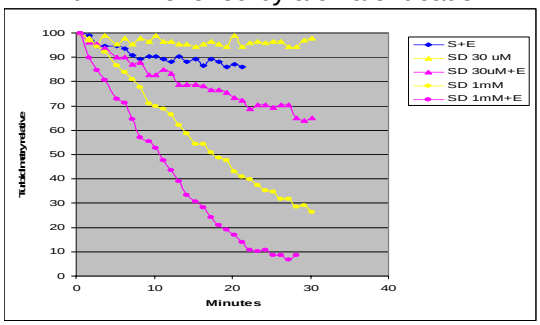
Zinc in the sperm nucleus

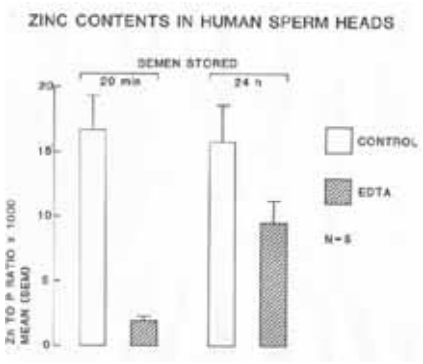
- Zinc is incorporated during spermiogenesis and the sperm chromatin contains one zinc for every protamine molecule (and theoretically) for each turn of the DNA-helix (=20 base-pairs) (Kjellberg, 1993).
- The zinc concentration is some 8 mM
- 90% of the zinc can be extracted close to ejaculation. This fraction diminishes upon storage.

Ward & Coffey 1991



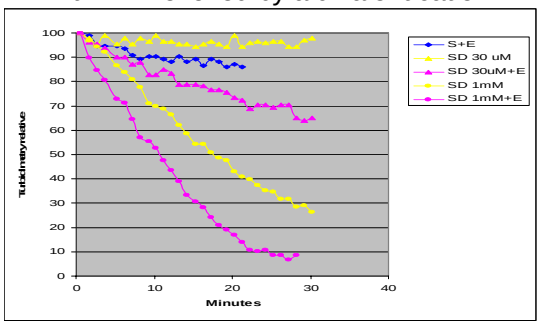
Major resistance towards decondensation with DTT evoked by a divalent cation





- **HP2** (thiol groups intact) can bind Zn(II)
- **In the presence of DNA**, the strongest Zn(II) binding is provided by sequences
- 12-22 (by His-12,Cys-13, His-19, and His-22) and
- 43-57 (His-43, Cys-47, Cys-53, and His-57)
- Zn(II) markedly **enhances DNA binding to protamine.** These findings suggest that Zn(II) ions may be a regulatory factor for sperm chromatin condensation processes.
- Bal et al. **Differential zinc and DNA binding by partial peptides of human protamine HP2.** Mol Cell Biochem. 2001 Jun;222(1-2):97-106

Major resistance towards decondensation with DTT evoked by a divalent cation



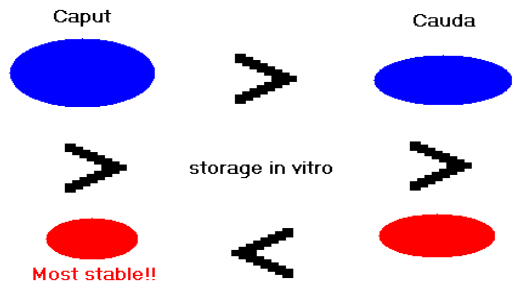
Does the increased resistance depend on S-S formation or the presence of zinc-thiol-bridges?

- Results from human ejaculated spermatozoa exposed to SDS + DTT+EDTA:
- The concentration of DTT can be reduced 50 times, from 1 mM to 30 uM if 6 mM EDTA is added to the SDS-DTT solution to obtain decondensation.

Conclusion: Most of the resistance towards sperm chromatin decondensation in vitro with DTT is dependent on the presence of Zinc.

During storage in cauda

- Prolonged in vitro storage of epididymal cauda sperm increase the resistance of spermatozoa towards decondensation in SDS-DTT (Calvin & Bedford 1971).
- Also caput epididymal sperm increase their reistance towards decondensation if experimentally delayed in the caput or if aged in vitro.
- Interestingly, these spermatozoa become the most resistant.



What does that mean?

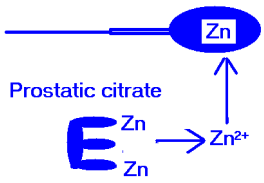
- 1) Cauda epididymal spermatozoa still have thiols that can be engaged in S-S bridge formation upon oxidation in vitro.
- 2) That caput spermatozoa become most resistant means that some thiols in the cauda sperm are hindered to take part in S-S oxidation. Hindered by ?, Zinc?

The ejaculatory sequence

- Man offers the woman spermatozoa in prostatic fluid

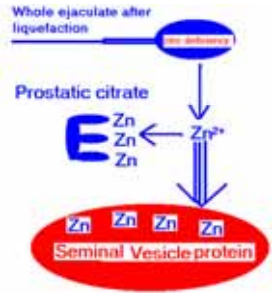
Chromatin zinc is retained by prostatic fluid

- The physiological ejaculate is spermatozoa suspended (emitted) in prostatic fluid and expulsed in the very first split-ejaculate fraction onto the cervix.
- Spermatozoa in prostatic fluid retain chromatin zinc.



Chromatin zinc is depleted by Seminal vesicular fluid

- Seminal vesicular fluid contains High molecular weight proteins (seminogelins) trapping zinc.
- HMW-Zn
- Increased pH increase the binding of zinc to citrate.



Liquefied ejaculate can act zinc-chelating,% HMW-Zn

- 20 fertile men 13% (Arver 1982)
- 13 fertile donors < 10% (Kjellberg, 1993)
- 115 infertile men 2-67% (Kjellberg 1993)

Liquefied whole ejaculate can act a zinc-chelating medium, especially in men with low seminal zinc concentration, indicating abundancy of seminal vesicular fluid

Vesicular fluid chelates chromatin zinc

- Spermatozoa expelled in vesicular fluid at ejaculation reveal lower zinc content in the chromatin (Björndahl, 1990).
- Spermatozoa incubated in seminal vesicular fluid loose zinc

Chromatin zinc

- Fertile donors have higher zinc content in chromatin than infertile men.
- Men with signs of prostatic inflammation had the lowest chromatin zinc content (Kvist, 1988).

Abnormal sequence - Spermatozoa expelled with vesicular fluid

- Men with low chromatin zinc were found to have an abnormal sequence of ejaculation with the majority of spermatozoa emitted in the first fractions dominated by fluid from the seminal vesicles.
- Thus a delayed emptying of the prostate due to inflammatory conditions could be one mechanism for this condition, another explanation could be a premature emission of seminal vesicular fluid (Björndahl, 1991).

Low seminal zinc halves pregnancy rate without affecting fertilization rate

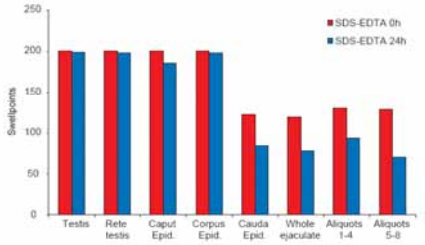
- indicates that the messages of the fertilizing spermatozoon was affected rather that its functions as a carrier of the message (Kvist, 2000).

Zn(mM)	N	% pregnancy trend $_{p<0.05}$.
<1.1	35	20.0
1.1-2.1	35	28.6
>2.1	36	41.7

- 106 consecutive first IVF-cycles with tubal occlusion and >= 3 oocytes retrieved.

Decondensation of boar spermatozoa in SDS-EDTA

Sum of moderately swollen x 1 anc and grossly swollwn x 2



Conclusions 1

- Sperm DNA is condensed and stabilized by protamines containing arginine that can neutralize negative charges of the DNA phosphates, cysteine that can form stabilizing bridges with zinc and disulfide-bridges, histidine that can bind zinc with cysteine and threonine and serine that can turn repulsively negative by phosphorylation.

Conclusions 2

- Condensation occurs mostly in the testis but a further stabilization occurs in the epididymis involving the cysteines and probably zinc.

Conclusions 3

- Zinc is incorporated during spermiogenesis and the sperm chromatin of ejaculated spermatozoa contains one zinc for every protamine molecule (and theoretically) for each turn of the DNA-helix (=20 base-pairs) (Kjellberg, 1993).
- Close to ejaculation 90% of spermatozoa reveal a zinc-dependent chromatin stability, i.e. sperm chromatin decondensation can be triggered by removal of zinc.
- After ejaculation, the inherent zinc-dependent stability is superseded by a zinc-independent stability involving oxidation of free thiols into disulphide bridges (Björndahl, 1985, Kvist, 1985).