Animal Science Papers and Reports vol. 21 (2003) no. 3, 167-181
Institute of Genetics and Animal Breeding, Jastrzębiec, Poland

Effects of decondensing agents on chromatin stability of boar spermatozoa – radioisotope study*

Jerzy Strzeżek, Władysław Kordan

Department of Animal Biochemistry and Biotechnology,
University of Warmia and Mazury in Olsztyn,
Oczapowskiego 5, 10-718 Olsztyn-Kortowo, Poland

(Received June 23, 2003; accepted August 25, 2003)

Chromatin stability is an important determinant of semen quality, essential for spermatozoa maturation in epididymes and early embryogenesis.
A radioisotope method based on the quantitative measurements of tritium-labelled actinomycin D (^{3}H-AMD) incorporation into the spermatozoa nuclei was used to assess chromatin stabilization of boar spermatozoa incubated with physiological (reduced glutathione – GSH, heparin – H and bovine serum albumin – BSA) or non-physiological (dithiothreitol – DTT, disodium ethylenediaminetetra acetate – EDTA, 2-mercaptoethanol – ME and sodium dodecyl sulphate – SDS) decondensing agents. The effect of the composition of seminal plasma and the role of zinc ions in chromatin stability of spermatozoa were also studied. Pre-treatment of spermatozoa with GSH, H, DTT, ME or SDS resulted in an increase in the incorporation of ^{3}H-AMD into the spermatozoa nuclei. In contrast, when sperm samples were treated with BSA or EDTA there was a reduction in the incorporation of ^{3}H-AMD, what was attributed to hyperstabilization of chromatin. A presumed hyperstabilization was also observed when SDS+EDTA+H were used. On the other hand, an exceptionally strong action of decondensation of chromatin was induced by H+BSA.
Increased incorporation of ^{3}H-AMD into the spermatozoa nuclei was concomitant with low zinc and protein content in the seminal plasma of boars following depletion test (DT), suggesting disturbances in chromatin stability. The presented radioisotope method based on the application of ^{3}H-AMD is a simple and reliable assay that can be used to monitor the chromatin status of boar spermatozoa.

KEY WORDS: actinomycin D (^{3}H-AMD) / boar / chromatin stability / decondensing agents / seminal plasma / spermatozoa

* Supported by the University of Warmia and Mazury in Olsztyn, project No. 0103.0803.

Changes in the chromatin stability are essential for maturation of mammalian spermatozoa, egg fertilization and embryonic development [Strzeżek 1998]. However, the molecular mechanisms responsible for the proper structure of spermatozoa chromatin are still unclear.

The disulphide bridges (S-S) between adjacent protamines, which are protected by the quarternary structure of the deoxyribonucleoprotein complex (DNP), play a major role in nuclear chromatin stabilization [Huret 1986]. Zinc ions (Zn^{2+}) can also play a special role in chromatin stabilization of spermatozoa of some mammalian species. It should be emphasized that the level of zinc ions in the sperm head is reduced during the transport of the sperm cells in the epididymis, which is associated with increased formation of disulphide bridges (S-S). Zinc ions may play a role in the formation of S-Zn-S bridges, which additionally stabilize the chromatin structure. Furthermore, the seminal plasma has been shown to play a specific role in the stabilization of Zn^{2+} ions level [Kvist and Eliasson 1980]. A former study by Kjellberg [1993] showed that disturbances in the secretory functions of the accessory sex glands, particularly boar seminal vesicles, affected chromatin stability and might result in male infertility. This phenomenon is dependent on the concentrations of specific protein ligands present in the seminal plasma, which regulate the levels of Zn^{2+} ions in the chromatin of spermatozoa, as well as its stability.

Reduced glutathione (GSH), heparin and albumin levels have been found in the secretions of a female reproductive tract and are physiological decondensing agents of sperm nuclei [Huret 1986, Reyes *et al.* 1989]. Reduced glutathione, a disulphide bridge-reducing agent, and heparin which binds specifically to the sperm plasmalemma, have been shown to induce nuclear decondensation of human spermatozoa [Reyes *et al.* 1989]. Moreover, albumin can induce membrane destabilization and sperm decondensation [Huret 1986]. The chromatin of spermatozoa is susceptible to induced decondensation. The decondensing agents regularly used are dithiothreitol (DTT), sodium dodecyl sulphate (SDS), disodium ethylenediaminetetra acetate (EDTA), glutathione (GSH) and 2-mercaptoethanol (ME) – Kvist [1982] and Reyes *et al.* [1989]. Binding of Zn^{2+} ions by some of these decondensing agents may contribute to the release of free thiol groups, which can destabilize the disulphide bridges (S-S) between protamines. Heparin shows strong affinity for sperm plasmalemma and ability to bind Zn^{2+} ions [Reyes *et al.* 1989].

At present, many methods are used to assess the degree of chromatin condensation. These include the flow cytometric analysis allowing a rapid assessment of the semen quality with the use of various fluorescent DNA-specific dyes [Gorczyca *et al.* 1993]. Recently, the "comet assay", also referred to as the single cell gel electrophoresis (SCGE) has been used to assess DNA integrity of mammalian spermatozoa. Denatured and fragmented DNA with single or double-strand breaks flows out of the sperm cell and moves towards the anode, causing the cell and its DNA to resemble a comet [Singh 2000].

The radioisotope assay is an established method for the assessment of the chroma-

tin status of spermatozoa. The method is based on the quantitative measurements of tritium-labelled actinomycin D (^{3}H-AMD), which has affinity for guanine-cytosine in the DNA structure [Darżynkiewicz *et al*. 1969, Balhorm *et al*. 1985]. The intensity of ^{3}H-AMD binding to sperm nuclei depends on the degree of chromatin condensation, the type of protein-forming complexes with DNA, and the morphological status of the sperm head [Ebstein 1972]. This study was aimed to investigate the effect of different incubation variants of decondensing agents (physiological and non-physiological) and to define the role of seminal plasma in chromatin stabilization of boar spermatozoa. The effect of depletion test (DT) on chromatin stability of boar spermatozoa was also studied.

Material and methods

Semen collection and spermatozoa preparation

Ejaculates were collected using the gloved-hand technique from three Polish Large White boars at the age of 3-5 years, kept in a climate-controlled environment and fed a commercial diet. Water was available *ad libitum*. The study was approved by the Local Ethics Commission. The official regulations (Directive on Animal Protection, Government Gazette of Republic of Poland, No. 111 item 724, August 21, 1997) on animal protection were followed.

After collection, the ejaculates were filtered through a sterile gauze, and centrifuged (12,500 × g, 15 min) at room temperature. After the supernatant was decanted, the sperm pellets were resuspended in 10 ml of 250 mM Tris-citric fructose buffer (pH 7.0) supplemented with tritium-labelled actinomycin D (^{3}H-AMD) (AMERSHAM INTERNATIONAL, Amersham, UK) with a radioactivity of 0.2 μCi/ml. The cells were resuspended by gentle agitation and transferred to glass tubes. Spermatozoa concentrations were determined cytometrically, their final concentration being 50×10^6/ml. The sperm samples supplemented with ^{3}H-AMD were used for radioisotope studies.

^{3}H-actinomycin D (^{3}H-AMD) binding to spermatozoa chromatin

Binding of ^{3}H-AMD to sperm chromatin was measured after Coulter [1973] with some modifications [Strzeżek *et al*. 1995]. Two variants of incubations of spermatozoa were used: without or with 200 IU heparin. One ml sperm samples were taken at 0, 30 and 60 min of incubation at 37°C. The samples were centrifuged (12,500 × g, 10 min), and 0.1 ml of the supernatant (three repeations) was placed in scintillation vials. Nine millilitres of the scintillation fluid were added to each sample, which was then thoroughly mixed for 24 h. Counts obtained from a liquid scintillation counter LS 5000 TD (BECKMAN) were corrected for the background and the appropriate dilution factor. Results are presented as the total counts taken up by 50×10^6 spermatozoa, expressed as a decrease of radioactivity (cpm) in the supernatants after 30 and 60 min incubation of the sample (with or without heparin) in relation to the 0-time sample.

Effect of different chromatin decondensing agents on ^{3}H-AMD binding

Sperm cells (50×10^6/ml) suspended in Tris-citric fructose buffer were alternatively incubated with 25 mM dithiothreitol (DTT, SERVA), 6 mM disodium ethylenediaminetetra acetate (EDTA, SIGMA), 10 mM 2-mercaptoethanol (ME, LOBA-CHEMIE, Wien-Fischamed), 1% sodium dodecyl sulphate (SDS, KOCH-LIGHT Laboratories Ltd., Colnbrook Bucks), 40 IU heparin (POLFA, Poland), 10 mM reduced glutathione (GSH, SIGMA) or 5% bovine serum albumin (BSA, SIGMA) for 1 h at 37°C. Following incubation, the ^{3}H-AMD solution with a radioactivity of 0.2 μCi/ml was added. The measurements of ^{3}H-AMD-binding to spermatozoa chromatin were conducted as already described. A combination of two or more decondensing agents was also analysed. A total of six radioisotope analyses were conducted for each incubation variant. The sperm samples without decondensing agents were used as a control.

Role of seminal plasma in chromatin stabilization of boar spermatozoa

Whole seminal plasma, zinc-free seminal plasma (2 h post-treatment with 3 mM EDTA), and the protein- and zinc-free fluid of electrolyte composition identical with seminal plasma (OLEP) – Larsson and Einarsson [1976] – containing 30 mM fructose (SIGMA), 50 mM sodium pyruvate (SERVA), 0.54 mM $CaCl_2$, 9 mM $MgCl_2$, 20 mM KCl, 60 mM NaCl and 10 mM $NaHCO_3$ (POLISH CHEMICAL REAGENTS, Gliwice, Poland) were used to investigate the effect of zinc ligands on decondensation of spermatozoa chromatin. Sperm pellets were prepared as described above. The suspended spermatozoa samples (50×10^6/ml) were incubated for 1h at 37°C with each incubation medium, as follows:

a) zinc-free seminal plasma + spermatozoa;
b) zinc-free seminal plasma with different concentrations of Zn^{2+} ions (0.9, 1.8, 2.7 mM respectively) + spermatozoa;
c) spermatozoa suspended in OLEP and incubated with 6 mM EDTA;
d) EDTA treatment of spermatozoa incubated with different concentrations of Zn^{2+} ions (0.9, 1.8, 2.7 mM, respectively);
e) whole seminal plasma + spermatozoa as a control.

The ^{3}H-AMD binding to the spermatozoa chromatin was measured as already described. Two incubation variants, without or with heparin (200 IU) were used.

Measurements of ^{3}H-AMD binding to spermatozoa chromatin during depletion test (DT)

Depletion test (DT) was based on the intensive sexual exploitation of boars for 10 consecutive days, during which semen was collected repeatedly until the boars refused to mount within 30 min from the preceding collection [Strzeżek *et al.* 2000]. The test determines the daily sperm output, reserves of the extra-gonadal spermatozoa and production of chemical components of the seminal plasma. The first three days (Period 1) reflected the reserves, whereas the remaining seven days (Period 2) represented the actual daily production of the chemical components. Spermatozoa chromatin stability obtained from Periods 1 and 2 of DT was assessed using ^{3}H-AMD as already described.

Heparin was used as the decondensing agent.

Biochemical analyses

Total protein content was determined after Lowry *et al*. [1951] using BSA as a standard. Zinc content was determined colorimetrically according to Lampugnani and Maccheroni [1984].

Statistical

Results were expressed as means and their standard deviations (SD), and significance of differences between means ($P \leq 0.05$) was evaluated according to Duncan's multiple range test. The relationship between some variables was evaluated using Pearson's correlation coefficients. Used was the STATISTICA for Windows, Inc. computer programme, Tulsa, USA.

Results and discussion

The phenomenon of spermatozoa chromatin decondensation is a natural physiological process, which accompanies egg fertilization. However, its mechanism is not fully understood, although disulphide-chelating or reducing agent is accepted as playing a major role. For example, Reyes *et al.* [1989] showed that 21.6 mM heparin and 5 mM GSH induced about 90% chromatin decondensation of human spermatozoa, following incubation for 1h at 37°C. Both heparin and GSH are components of the secretory fluid of the female reproductive tract, including the oviduct. Moreover, heparin is a component of glycosoaminoglycans (GAG) synthesized by the granulosa and thecal cells [Strzeżek 1998], whereas GSH is present in ovulated oocytes.

There was a gradual increase in radioactivity when spermatozoa were incubated with the majority of decondensing agents used, depening on the incubation time and decondensing agent, as shown in Figure 1. Heparin, ME, GSH, SDS and DTT induced chromatin decondensation, as shown by increased ^{3}H-AMD binding. The reduced ^{3}H-AMD binding may reflect hyperstabilization of chromatin shown by BSA and EDTA, a chelator of Zn^{2+} ions. Similar effects were shown when two or more decondensing agents were used simultaneously in the incubation media (Fig. 2). Heparin inhibited the action of non-physiological decondensing agents – DTT, SDS and ME. Interestingly, heparin suppressed hyperstabilization induced by BSA that is known to induce sperm capacitation related to the removal of Zn^{2+} ions from the sperm cells. It may be suggested, that when both heparin and BSA bind specifically to the plasmalemma, chromatin decondensation is induced. Special attention should also be devoted to the hyperstabilization of chromatin in spermatozoa treated with SDS + EDTA. SDS causes membrane disruption, whereas EDTA acts as a chelating agent. The lack of disulphide-reducing agent in the chromatin structure, disturbance in the integrity of heparin receptors on the plasmalemma and the premature binding of Zn^{2+} ions by EDTA may lead

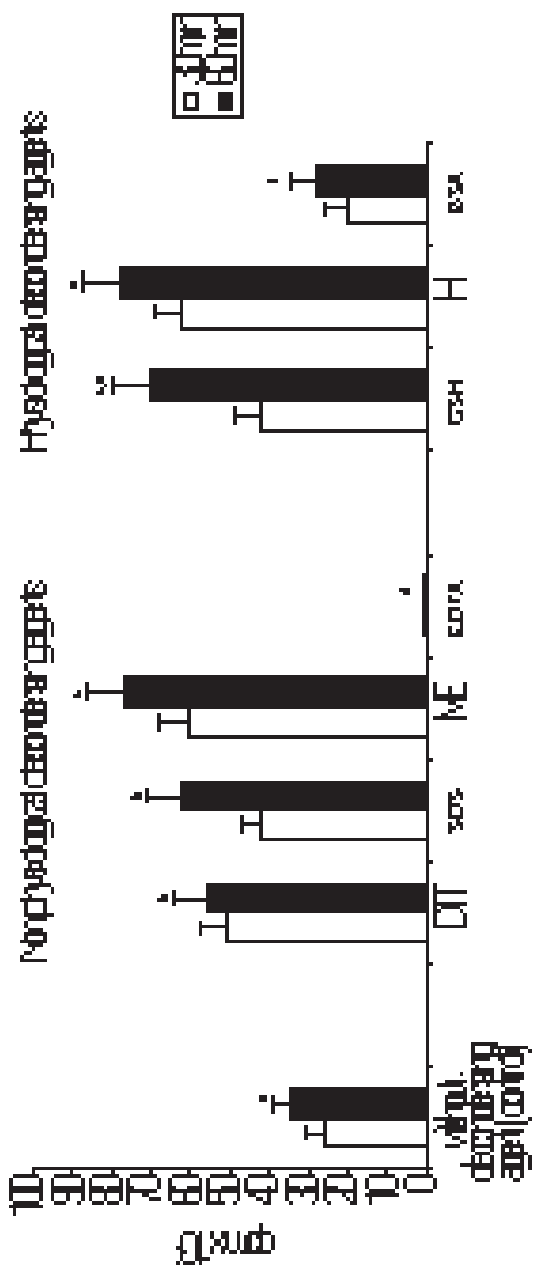

Fig. 1. Effect of non-physiological and physiological decondensing agents on ^{3}H-AMD binding to chromatin of boar spermatozoa (means and their SDs). Means marked with different letters are significantly different at $P \leq 0.05$ (60 min of incubation).

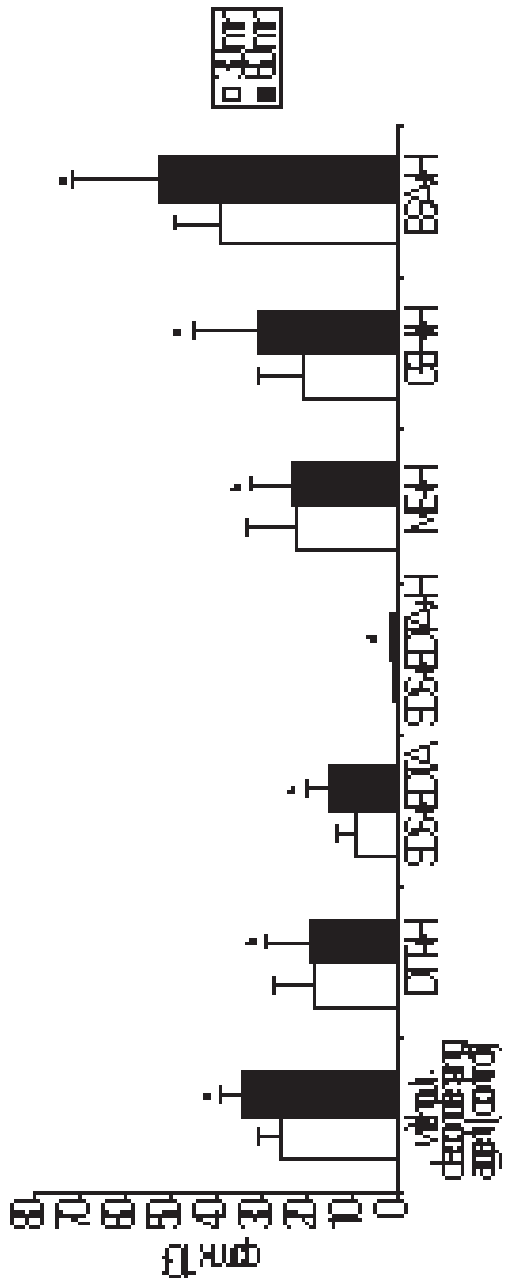

Fig. 2. Effect of combined decondensing agents on ^{3}H-AMD binding to chromatin of boar spermatozoa (means and their SDs). Means marked with different letters are significantly different at P≤0.05 (60 min of incubation).

to inadequate chromatin decondensation in the incubation medium containing SDS + EDTA in the presence of heparin.

The seminal plasma, and particularly its proteinous components, play a special role in the spermatozoa chromatin stabilization [Strzeżek 1999]. According to Kjellberg [1993], high-molecular weight proteins secreted by the seminal vesicles have a strong affinity for Zn^{2+} ions, which may cause chromatin destabilization of human spermatozoa. Also, prostate secretion rich in Zn^{2+} ions, free or bound with low-molecular components, can act as a modulator of chromatin condensation.

As shown in Figure 3, introducing of 3 mM EDTA to whole seminal plasma did not produce any significant effect on ^{3}H-AMD binding to the chromatin of spermatozoa following 60 min incubation with heparin. Furthermore, the addition of different concentrations of Zn^{2+} ions to the incubation media significantly reduced chromatin susceptibility to heparin-induced decondensation, what was manifested by a marked reduction in ^{3}H-AMD binding. Also, a gradual reduction in the chromatin susceptibility to heparin-induced decondensation was observed when the spermatozoa were treated with OLEP and 6 mM EDTA (Fig. 4). However, similar effects were not observed when OLEP + EDTA were used without heparin. Introducing the increasing concentrations of Zn^{2+} ions to the incubation medium containing OLEP and EDTA caused a regeneration of the chromatin decondensation ability, as inferred from the increase in ^{3}H-AMD binding in the presence of heparin (Fig. 4). This indicates a key role that Zn^{2+} ions and protein ligands of the seminal plasma play in the maintenance of the chromatin stability of boar spermatozoa.

Intensive sexual exploitation may cause disturbance in the normal organization of nuclear chromatin of spermatozoa. Glogowski *et al.* [1994] demonstrated that spermatozoa from individual bulls ejaculated eight times weekly exhibited lower ^{3}H-AMD binding to the chromatin than those from bulls ejaculated four or six times a week. Earlier a gradual reduction was shown in heparin ability to induce chromatin decondensation of spermatozoa collected from intensively sexually exploited boars [Strzeżek *et al.* 1995].

Depletion test (DT) caused disturbance in the secretion of boar accessory sex glands, particularly the seminal vesicles, manifested by a drastic reduction in the protein (Fig. 5) and Zn^{2+} ions (Fig. 6) content of the seminal plasma. The changes were more evident during Period 2 of DT (days 4 to 10). Low protein and zinc content of the seminal plasma was synchronized with a reduction in ^{3}H-AMD binding to the spermatozoa chromatin (Tab. 1). High level of radioactivity associated with increased ^{3}H-AMD binding was observed during days 1 to 3 of depletion test (Tab. 1), which represented the reserves of the total sperm output In contrast, there was a marked reduction in ^{3}H-AMD binding during days 4 to 10, which represented the daily production of new sperm cells. The changes in ^{3}H-AMD binding were found significant only in the presence of heparin after 60 min incubation (Tab. 1). Binding of ^{3}H-AMD to the spermatozoa chromatin was negatively correlated with sperm concentration and zinc content and positively with total protein content of the seminal plasma, with no reference to the incubation

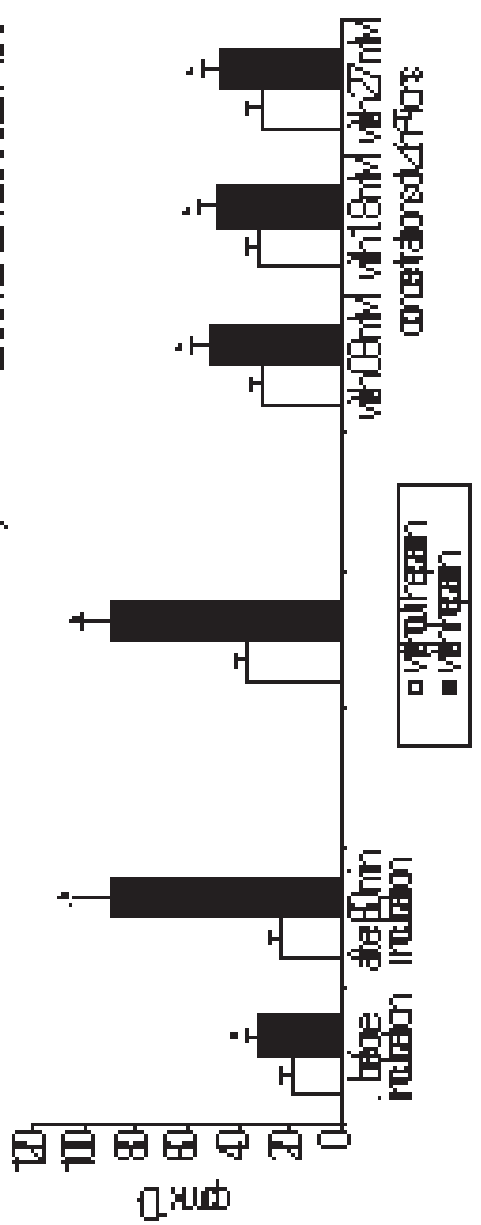

Fig. 3. Changes in ^{3}H-AMD binding to chromatin of boar spermatozoa following incubation with seminal plasma (means and their SDs). Means marked with different letters are significantly different at $P \leq 0.05$ (incubation with heparin).

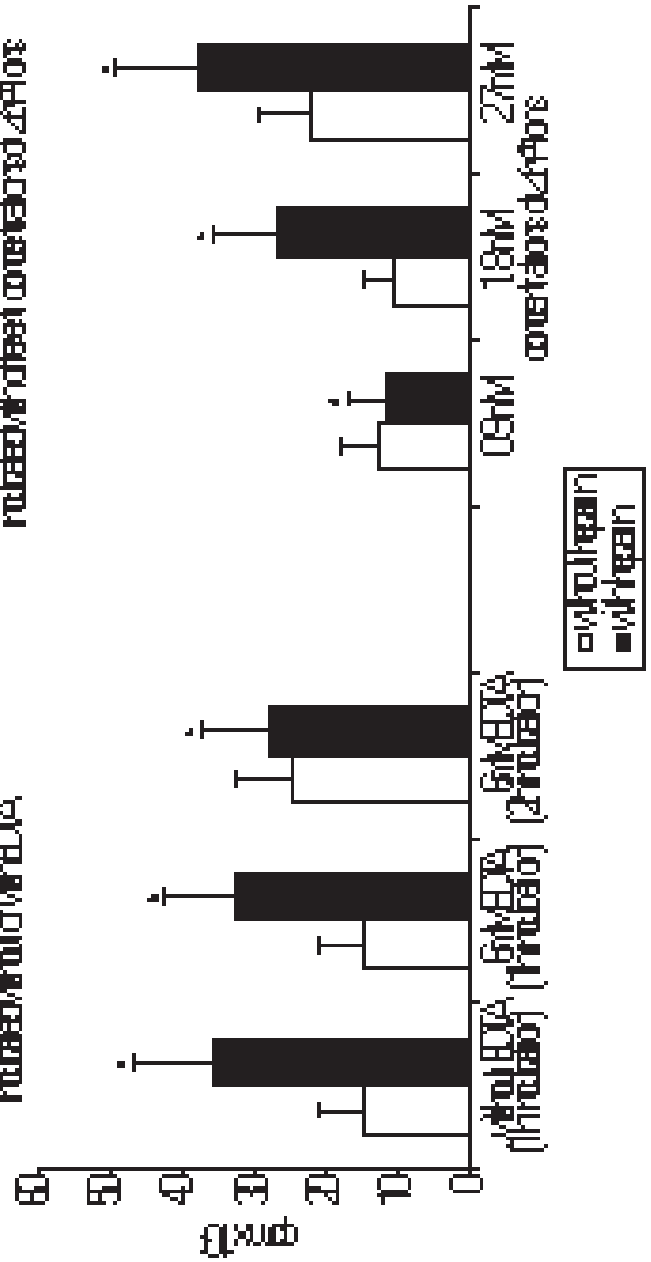

Fig. 4. Changes in ^{3}H-AMD binding to chromatin of boar spermatozoa suspended in OLEP and incubated with EDTA or Zn^{2+} (means and their SDs). Means marked with different letters are significantly different at $P \leq 0.05$ (incubation with heparin).

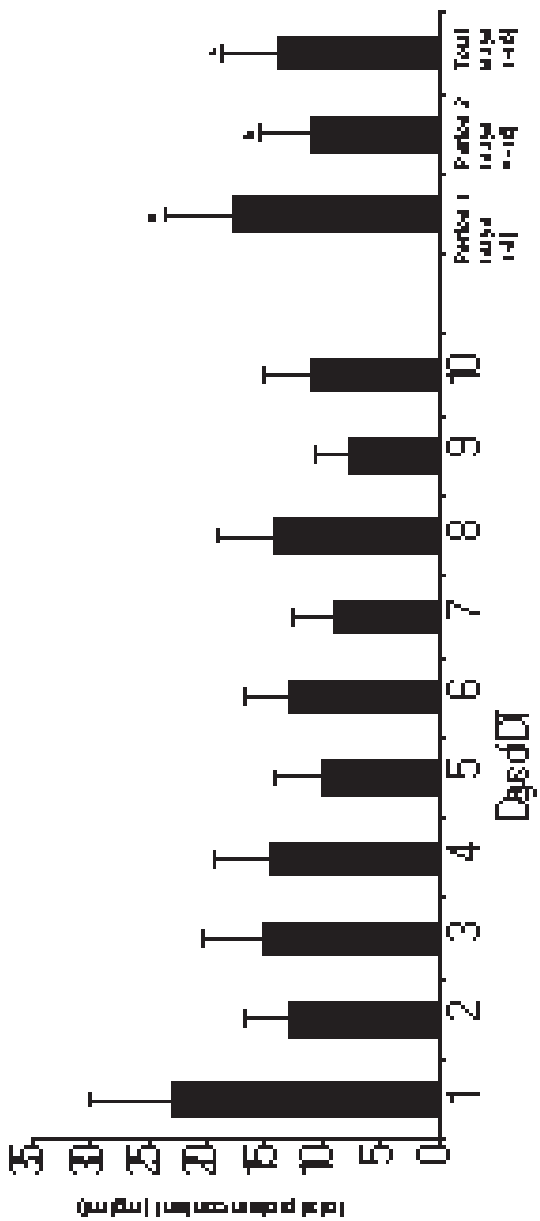

Fig. 5. Total protein content of seminal plasma of boars subjected to depletion test (DT) – means and their SDs. Means for periods marked with different letters are significantly different at P≤0.05.

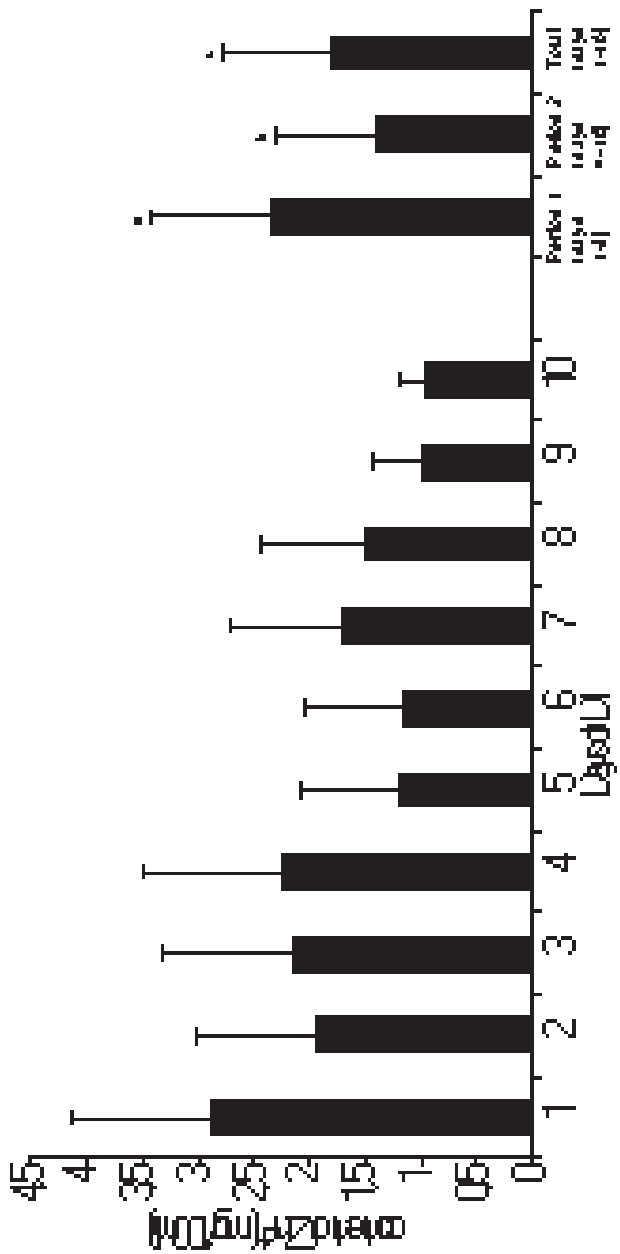

Fig. 6. Content of zinc ions in seminal plasma of boars subjected to depletion test (DT) – means and their SDs). Means for periods marked with different letters are significantly different at P≤0.05.

Table 1. Effects of depletion test (DT) on ^{3}H-AMD binding to chromatin of boar spermatozoa as related to incubation time and decondensing agent

DT	Day	No. of ejaculates	^{3}H-AMD binding (cpm × 10^{3})							
			with heparin				without heparin			
			30 min		60 min		30 min		60 min	
			mean	SD	mean	SD	mean	SD	mean	SD
Period 1	1-3	21	32.75	13.87	42.59[a]	1.17	17.65	8.48	26.62	10.00
Period 2	4-10	35	26.41	10.11	32.37[b]	11.56	15.58	7.63	25.52	7.92
Total	1-10	56	28.60	11.82	32.20[b]	14.83	17.10	8.60	22.35	9.35

[a,b]Within column means bearing different superscripts differ significantly at $P \leq 0.05$.

Table 2. Correlation coefficients between ^{3}H-ADM binding to chromatin of boar spermatozoa and selected parameters of semen

Semen parameter	^{3}H-ADM binding	
	30 min	60 min
Sperm concentration	-0.40**	-0.38**
Total protein content	0.35*	0.38*
Zinc content	-0.39**	-0.30*

*$P \leq 0.05$; **$P \leq 0.01$.

time (Tab. 2).

The results presented here indicate that decondensing agent such as heparin and BSA may play a physiological role by inducing decondensation of nuclear chromatin of boar spermatozoa during fertilization. This indicates that Zn^{2+} ions and their protein ligands may act as modulators of the chromatin status of boar spermatozoa. Furthermore, depletion test confirmed that disturbances in the secretion of seminal plasma components can directly affect the spermatozoa chromatin susceptibility to decondensing agent. Since the sensitivity of chromatin decondensation depends on the physiological mechanisms, its disturbance may lead to the infertility of males.

Acknowledgement. *The authors thank Dr Leyland Fraser for kindly reviewing the manuscript.*

REFERENCES

1. BALHORM R., KELLARIS K., CORZET M., CLANCY C., 1985 – 7-aminoactinomycin D binding and final stages of sperm chromatin. ***Gamete Research*** 12, 411-422.
2. COULTER G.H., 1973 – Biochemical and physical changes in bovine spermatozoa due to freezing at –196°C and age of bull. Thesis. Cornell University, 1-122.
3. DARŻYNKIEWICZ Z., GLEDHIL B.L., RINGERTZ N.R., 1969 – Changes in deoxyribonucleoprotein during spermatogenesis in the bull: ^{3}H–actinomycin D binding capacity. ***Experimental Cell Research*** 56, 435-438.
4. EBSTEIN B.S., 1972 – Tritiated actinomycin D as a cytochemical label for small amounts of DNA. ***Journal of Cell Biology*** 53, 709-714.
5. GLOGOWSKI J., STRZEŻEK J., JAŻDŻEWSKI J., 1994 – Intensity of ^{3}H–Actinomycin (^{3}H-AMD) binding to chromatin of bull spermatozoa. ***Reproduction in Domestic Animals*** 29, 396-403.
6. GORCZYCA W., TRANANOS F., JESIONOWSKA H., DARŻYNKIEWICZ Z., 1993 – Presence of DNA strand breaks and increased sensitivity of DNA in situ to denaturation in abnormal human sperm cells, analogy to apoptosis of somatic cells. ***Experimental Cell Research*** 207, 202-205.
7. HURET J.L., 1986 – Nuclear chromatin decondensation of human sperm: a review. ***Archives of Andrology*** 16, 97-109.
8. KJELLBERG S., 1993 – Zinc and human sperm chromatin. Linkoping University, Medical Dissertations, 400, 5-69.
9. KVIST V., 1982 – Spermatozoal thiol-disulphide interaction: A possible event underlying physiological sperm nuclear chromatin decondensation. ***Acta Physiologica Scandinavica*** 115, 503-505.
10. KVIST V., ELIASSON R., 1980 – Sperm nuclear chromatin decondensation ability. ***Acta Physiologica Scandinavica***109 (Supplement) 486, 1-24.
11. LAMPUGNANI L., MACCHERONI M., 1984 – Rapid colorimetry of zinc in seminal fluid. ***Clinical Chemistry*** 30 (8), 1366-1368.
12. LARSSON K., EINARSSON S., 1976 – Fertility of deep frozen boar spermatozoa. Influence of thawing diluents and boars. ***Acta Veterinaria Scandinavica*** 17, 43-62.
13. LOWRY I.O.M., ROSENBROUGH N.I., FARR R.L., RANDALL R.J., 1951 – Protein measurement with folinphenol reagent. ***Journal of Biological Chemistry*** 193, 265-275.
14. REYES R., ROSADO A., HERNANDEZ O., DELGADO N.M., 1989 – Heparin and glutathione: Physiological decondensing agents of human sperm nuclei. ***Gamete Research*** 23, 39-47.
15. SINGH N.P., 2000 – Microgels for estimation of DNA strand breaks DNA protein crosslinks and apoptosis. ***Mutation Research*** 455, 111-127.
16. STRZEŻEK J., 1998 – Fizjologia i biochemia struktur plemnika ssaków. W: Ultrastruktura i funkcja komórki, mechanizmy regulujące spermatogenezę (Physiology and biochemistry of structures of mammalian spermatozoa. In: Ultrastructure and function of a cell and spermatozoa regulating mechanisms). In Polish. Wydawnictwo Naukowe PWN, Warszawa 7, 99-126.
17. STRZEŻEK J., 1999 – Plasma nasienia a niektóre biologiczne funkcje plemników (Seminal plasma and some biological functions of spermatozoa). In Polish with English summary. ***Postępy Biologii Komórki*** 26 (Supplement) 12, 59-68.
18. STRZEŻEK J., KORDAN W., GLOGOWSKI J., WYSOCKI P., BORKOWSKI K., 1995 – Influence of semen-collection frequency on sperm quality in boars, with special reference to biochemical markers. ***Reproduction in Domestic Animals*** 30, 85-94.
19. STRZEŻEK J., FRASER L., DEMIANOWICZ W., KORDAN W., WYSOCKI P., HOŁODY D., 2000 – Effect of depletion tests (DT) on the composition of boar semen. ***Theriogenology*** 54, 949-963.

Jerzy Strzeżek, Władysław Kordan

Wpływ czynników dekondensujących na stabilizację chromatyny plemników knura – badania izotopowe

S t r e s z c z e n i e

Stabilność chromatyny plemników jest istotnym wyznacznikiem jakości nasienia, bowiem determinuje przebieg procesu dojrzewania plemników w najądrzach oraz wczesny rozwój zarodkowy.

W badaniach zastosowano radioizotopową metodę pomiaru wiązania ^{3}H-aktynomycyny D (^{3}H-AMD) przez jądra plemników inkubowanych z czynnikami dekondensującymi chromatynę: fizjologicznymi (zredukowany glutation – GSH, heparyna – H, albumina surowicy bydlęcej – BSA) i niefizjologicznymi (ditiotreitol –DTT, wersenian dwusodowy – EDTA, merkaptoetanol – ME, laurylosiarczan sodowy – SDS). Badano wpływ składu plazmy nasienia na stabilność chromatyny plemników, ze szczególnym uwzględnieniem funkcji jonów Zn^{2+}. W przypadku zastosowania GSH, H, DTT, ME i SDS stwierdzono wzrost wiązania ^{3}H-AMD przez chromatynę plemników. Po inkubacji plemników z BSA lub EDTA obserwowano spadek wiązania. Pod wpływem grupy czynników dekondensujących w mieszaninie inkubacyjnej: SDS-EDTA-H, w nieobecności DTT (specyficznie rozszczepiającego mostki -S-S-) obserwowano spadek wiązania ^{3}H-AMD, co zinterpretowano jako hyperstabilizację chromatyny. Silne działanie dekondensujące chromatynę przez kompleks czynników fizjologicznych BSA-heparyna, uznano za skutek ich działania jako układu ligandów wiążących Zn^{2+}, co sprzyjało destabilizacji wiązań S-Zn-S.

Dziesięciodniowe próby opróżniania knurów, czemu towarzyszyło stopniowe obniżanie produkcji plemników i zdolności sekrecyjnej dodatkowych gruczołów płciowych, były sprzężone ze spadkiem wiązania ^{3}H-AMD. Wyraźne obniżenie zawartości białka całkowitego i koncentracji jonów Zn^{2+} w plazmie niasienia obserwowane podczas prób opróżniania podkreśla udział tych komponentów w stabilizacji chromatyny plemników knura.

Zastosowana metoda radioizotopowa z użyciem ^{3}H-AMD może być wykorzystana w biochemicznej ocenie stanu chromatyny plemników.