Utilization of aminoguanidine prevents cytotoxic effects of semen

Mirja Harms
University Ulm Medical Center
Pascal von Maltitz
University Ulm Medical Center
Benjamin Mayer
Ulm University
Miriam Deniz
Ulm University Hospital
Janis Müller
Philipps University of Marburg
Jan Münch (jan.muench@uni-ulm.de)
University Ulm Medical Center

Article

Keywords:

Posted Date: May 17th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1624893/v1

Abstract

Studies of human semen in cell or tissue culture are hampered by the high cytotoxic activity of this body fluid. Responsible for the cell damaging activity of semen are amine oxidases, which convert abundant polyamines such as spermine or spermidine in seminal plasma into toxic intermediates. Amine oxidases are naturally present at low concentrations in seminal plasma and at high concentrations in fetal calf serum, a commonly used cell culture supplement. We here show that in the presence of fetal calf serum, seminal plasma as well as the polyamines spermine and spermidine are highly cytotoxic to immortalized cells, primary blood mononuclear cells, and vaginal tissue. Thus, experiments investigating the effect of polyamines and seminal plasma on cellular functions should be performed with great caution, considering confounding cytotoxic effects. Addition of the amine oxidase inhibitor aminoguanidine to fetal calf serum and/or utilization of serum-free medium greatly reduced this serum-induced cytotoxicity of polyamines and seminal plasma in cell lines, primary cells and tissues, and thus should be implemented in all future studies analyzing the role of polyamines and semen on cellular functions.

Introduction

Human semen (SE) is a complex body fluid consisting of a cellular fraction of mainly spermatozoa, and a cell-free fraction, the seminal plasma (SP). An average human ejaculate has a volume of 2–6 ml, consists of up to 10% spermatozoa and 90% SP, and has a pH in the range of 7.2–8.0.[1] SP originates from the accessory organs of the male reproductive tract and its main function is to provide a protective and feeding environment for spermatozoa and to counteract the acid vaginal environment during fertilization.[2] Studies of SE and SP are complicated by intra- and inter donor variations, as well as time-, age- and diet-related changes in the composition.[3–5] Moreover, fresh gel-like ejaculates undergo a rapid 15–30 min protease-mediated liquefaction process which results in the liberation of motile spermatozoa. Therefore, studies with SE or SP are challenging and require well-defined standard operation procedures of ejaculate collection, sampling, and storage, and should include the examination of many individual as well as pooled samples.

Studies investigating the effect of SE on cellular functions are further hampered due to the long-known but poorly studied cytotoxic activity of this body fluid.[6–11] Even 10- to 100-fold dilutions of SE or SP can induce cytotoxic effects in mammalian cell cultures,[12–14] which precludes or complicates the analysis of physiologically relevant concentrations. Experiments with SP therefore require the establishment of experimental conditions that on the one hand exclude cell-damaging effects of the body fluid, e.g. through dilutions or limited incubation times, but on the other hand still allow the determination of the effect of SP on cellular parameters. Examples of such experiments are studies on the influence of SP on the infectivity of sexually transmitted viruses such as HIV-1 or on the anti-inflammatory properties of SP in the presence of immune cells.[12–16]

Mainly responsible for the cytotoxic effect of semen are the polyamines spermidine and spermine.[11,17–19] These small organic molecules are present in SP at high levels, with spermidine reaching concentrations

of up to 0.6 mM and spermine of 0.25 to 14 mM.[20,21] Both polyamines carry positive charges that mediate strong interactions with negatively charged molecules such as phosphate ions, nucleic acids and phospholipids. Spermine and spermidine are oxidized by amine oxidases in SP but also fetal calf serum (FCS), leading to the formation of aldehyde intermediates, acrolein and ammonia.[22–24] The formation of acrolein represents the most important toxic intermediate,[19,25] as this molecule interacts strongly with deoxyguanosine in DNA, triggering base substitutions, and with nucleophilic sites in proteins (e.g. cysteine, lysine, arginine and histidine), leading to cross-linking, changes in signaling pathways and enzymatic activities,[25] and ultimately to cell death. FCS is widely used as a supplement for growth media in eukaryotic cell culture and naturally contains amine and diamine oxidases.[24] Thus, amine oxidases present in FCS-supplemented cell culture produce toxic acrolein from polyamines, explaining the high cytotoxicity of SP.[22]

Consequently, blocking the activity of these enzymes that lead to polyamine oxidation and subsequent deamination should prevent acrolein production and may allow to perform cell culture experiments in the presence of physiologically relevant concentrations of polyamines and/or semen. Aminoguanidine (AG), an amino derivate of guanidine was shown to strongly inhibit Cu^{2+}-dependent amine oxidases, including diamine and serum amine oxidases.[24,26–28] AG has a high affinity for diamine oxidases (apparent dissociation constant, K'1 = 0.7 nM)[29] most probably due to stabilization by conformational changes or formation of a Schiff base with the catalytic center of the enzyme.[29] AG has safely been used as cell culture supplement to prevent cytotoxic effects of spermine and spermidine .[30,31] Thus, we here evaluated whether addition of AG may also reduce or prevent the cytotoxic effects of SP in cell culture.

Results And Discussion

We first verified the cytotoxic activity of SE and SP. For this, pooled SE or SP derived from 50 donors was titrated on TZM-bl cells, a HeLa cell derivative widely used in AIDS research that is engineered to express the HIV-1 receptors CD4 and CCR5.[32] TZM-bl cells were seeded at a low cell density and treated with increasing SE or SP concentrations in DMEM supplemented with 10% (v/v) heat-inactivated FCS. After two days of incubation at 37°C, cell viability was determined using a colorimetric MTT assay (Fig. 1a) and light microscopy (data not shown). Microscopic evaluation revealed that cells treated with SE as well as SP concentrations as low as ~ 1% already exhibited a spherical shape, which became more evident with higher concentrations of the body fluids. At concentrations of 10% and higher, the cells detached completely from the bottom of the cell culture plate. The MTT-assay measures the conversion of a soluble MTT salt into a purple-colored formazan product in viable cells.[33] The MTT assay confirmed these observations and showed a concentration-dependent decrease in viable cells (Fig. 1a), as previously shown.[30,31] SE or SP concentrations > 1% led to an almost complete loss of viable cells and concentrations as low as 0.3% already resulted in a 20% reduction of viability (Fig. 1a). Thus, SE as well as SP are highly cytotoxic under standard cell culture conditions involving utilization of 10% FCS, confirming previous results of others and us. [6–14,17–19]

Next, we determined the cytotoxic activity of synthetic spermine and spermidine using the same experimental setup as described above. MTT assays performed after 2 days showed that in the presence of 10% FCS, concentrations of ≥ 10 µM of both polyamines were strongly cytotoxic for TZM-bl cells (Fig. 1b), confirming previous data obtained with several cell lines such as A549 lung adenocarcinoma and HCT116 colon adenocarcinoma cells,[30,31] human fibroblasts,[24] rat neurons,[34] or murine lymphocytes.[11] To quantify the polyamine concentrations in the pooled SP sample, liquid chromatography-tandem mass spectrometry was applied,[35] revealing concentrations of ~ 3 mM spermine, 155 µM spermidine, 80 µM putrescine and 255 µM L-ornithine (Fig. S1), in line with published data.[20] Thus, the spermine concentrations in SP are in a range that can explain the cytotoxicity of SP and suggests that spermine is the major contributor to the cell-damaging activity in semen.

To further investigate the role of serum in the observed effects, we titrated FCS on TZM-bl cells supplemented with PBS, 100 µM spermine (Fig. 1c) or 10% SP (Fig. 1d), and determined cell viability two days later. As expected, FCS alone had no effect on cell viability (Fig. 1c). Similarly, in the absence of FCS, spermine did not result in measurable cytotoxic activity (Fig. 1c). However, addition of only 1% FCS to cells exposed to spermine resulted in complete cell death (Fig. 1c). Similar results were obtained for 10% SP, which already caused 50% cell death in the absence of FCS, probably due to oxidases produced by the cells or naturally present in SP (Fig. 1d). These results suggest that spermine toxicity is dependent on the presence of FCS and that serum-free conditions may restore cell viability in the presence of otherwise toxic spermine concentrations.

We then analyzed the cytotoxicity of spermidine and spermine under serum-free cell culture conditions. For this, cells were either supplemented with a chemically-defined serum-free medium ("- serum" condition) or 10% FCS as control ("+ serum"), and then exposed to serial dilutions of spermidine and spermine. MTT tests performed 1, 2, and 3 days later confirmed strong cytotoxic effects of both polyamines at concentrations of ≥ 100 µM in the presence of 10% FCS (Fig. 2a, b). In contrast, under serum-free conditions, spermidine and spermine showed no strong cytotoxic effects at concentrations up to 5 mM, and reduced metabolic activity was only detected at the highest tested concentration of 10 mM (Fig. 2a, b). Thus, avoidance of FCS and utilization of serum-free medium allows studying spermine and spermidine at concentrations almost equivalent to those in semen.

To examine if amine oxidases in FCS are responsible for the conversion of spermine and spermidine into toxic intermediates, we analyzed the effect of the oxidase inhibitor AG. Control experiments showed that AG alone did not exert cytotoxic effects at concentrations of up to 5 mM in TZM-bl cells and primary blood mononuclear cells (PBMC) (Fig. S2), confirming previous data obtained with AG in other cells.[17,24,26,34] We then incubated 100% FCS with 0, 50, 500 and 5,000 µM of AG for 24 hours and supplemented TZM-bl cells with 10% (v/v) of these samples together with spermidine and spermine concentrations of up to 10 mM. Cells were then incubated and MTT assays performed 2 days later. This experiment showed that AG concentrations of 50, 500 and 5,000 µM effectively reverted FCS-mediated cytotoxicity of both polyamines (Fig. 3a, b). These results confirm that amine oxidases in FCS are

responsible for generating toxic polyamine products, as previously suggested.[11,17–19] Furthermore, we confirm AG as supplement that enables analysis of high spermine and spermidine concentrations in the presence of FCS.

We were suspecting that the use of chemically-defined serum-free medium and/or AG-treated FCS may not only prevent cytotoxic effects of spermine and spermidine, but also SP. To test this, TZM-bl cells were either incubated with the standard supplement of 10% FCS (FCS), AG-treated FCS (FCS preincubated with 0.5 mM AG), chemically-defined medium (no FCS), or AG-treated chemically defined medium (no FCS, 0.05 mM AG). Cells were then exposed to SP concentrations of up 40% (v/v) and cell viability was determined 2 days later by MTT assay. As shown in Fig. 4a, in the presence of FCS, SP was strongly cytotoxic at concentrations of 0.3% and higher, as expected (Fig. 1a). When cells were supplemented with AG-treated FCS, SP was less toxic, with still more than 90% viable cells in the presence of up to 10% of SP (Fig. 4a). Serum-free conditions also allowed to analyze SP at concentrations of up to 2.5% (Fig. 4a). A combination of both, serum-free medium and AG, prevented toxic effects of SP most efficiently, allowing the analysis of SP concentrations of up to 10 volume% without causing any significant reduction in cell viability (Fig. 4a). Similar results were obtained using primary PBMCs instead of immortalized cells (Fig. 4b). In the presence of serum, SP caused massive cell death after 3 days, even at concentrations of only 0.2% SP (Fig. 4b). Utilization of AG-treated FCS largely prevented toxicity, allowing to study SP at concentrations of up to 10% in PBMCs. Again, a combination of serum-free medium and AG most effectively reduced SP-induced toxicity (Fig. 4b).

Finally, we evaluated whether addition of AG and utilization of serum-free medium may allow preventing SP toxicity in vaginal tissue blocks. For this, tissues derived from 4 donors were dissected into 2 x 2 x 1 mm^3 blocks, and 10 blocks per donor were cultivated in either medium containing 10% FCS or serum-free medium containing 0.05 mM AG. Blocks were then exposed for three days to buffer or 10% and 20% SP (v/v) and cell viability was determined by measuring intracellular ATP levels using a luminescence-based assay (Fig. 5, Fig. S3), including a detergent control (0.5% triton). In the presence of FCS, 10% and 20% SP resulted in average in an 80% and 90% reduced viability as compared to the buffer control. Addition of AG completely rescued tissue viability in the presence of 10% SP and reduced viability by 25% in the presence of 20% SP. Thus, supplementation of medium with AG-treated FCS allows analysis of SP under conditions not affecting cell viability of vaginal tissue.

Conclusions

Our data confirm and expand those of others showing that 1) spermine and spermidine are cytotoxic in the presence of bovine serum[11, 17–19,34] and 2) that the amine oxidase inhibitor AG reduces toxicity of polyamines in the presence of FCS.[17,24,26,34] Moreover, we show that supplementation of FCS containing medium with AG allows to minimize the cytotoxic activity of seminal plasma, suggesting that amine oxidases present in FCS are the responsible factors for the cytotoxic activity of seminal plasma. These enzymes oxidize spermine and spermidine thereby producing harmful byproducts such as hydrogen

peroxide, ammonia, and reactive aldehydes.[19,22,23,26] Alternatively, utilization of serum-free medium supplemented with defined nutrients also allows to minimize the cytotoxic activity of polyamines and/or SP. We furthermore demonstrate that supplementation of FCS-containing medium with AG or utilization of chemically-defined serum allows investigation of SP in primary PBMC and vaginal tissues blocks. This is of particular importance as many studies reported an immunosuppressive activity of SP,[7,9,21,36,37] which were later questioned and attributed to misinterpretation of data due to overlooked cytotoxic effects of SP.[17,30] Similarly, the effect of semen and seminal plasma on the infectivity of sexually transmitted viruses is highly controversial, with enhancing or inhibiting effects of SP reported even for the same viral pathogen.[12–14,38–41] Thus, adaption of the protocols described herein, will foster future research to clarify the role of SP (and whole semen) on cellular process and viral infections in the recipient's tissues.

Methods

Reagents

Spermine and spermidine were purchased from Sigma Aldrich (#S3256 and #S2626). Aminoguanidine was obtained from Sigma Aldrich (#109266). Xvivo-15 growth medium was purchased from Biozym (#BE02-060F). DMEM and PRMI 1640 growth medium were purchased from Life Technologies (#41965-039 and ##21875-034). Fetal calf serum war purchased from Invitrogen (#10270106).

Semen and seminal plasma

Semen was provided by the "Kinderwunsch-Zentrum Ulm", a fertility center in Ulm. Semen of about 50 individual donors, was allowed to liquefy for 30 min, then pooled and stored in aliquots at -80°C. Seminal plasma represents the cell free supernatant of semen and was prepared by centrifugation at 20,000 × g for 30 min at 4°C.

Cell culture and primary tissue

Adherent TZM-bl (HeLa based) cells were obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH: TZM-bl cells (Cat#8129) from Dr. John C. Kappes, and Dr. Xiaoyun Wu and were grown in DMEM supplemented with 10% FCS, 2 mM L-glutamine, 100 units/ml penicillin and 100 µg/ml streptomycin. Cells were checked for mycoplasma contamination on regular basis. Human peripheral blood mononuclear cells (PBMCs) from healthy donors were prepared by Ficoll density centrifugation and activated for three days in RPMI medium containing 10 % FCS, 100 units/ml penicillin and 100 µg/ml streptomycin, 1 mM L-glutamine, 10 ng/ml interleukin-2 (IL-2) and 1 µg/ml phytohemagglutinin (PHA). Blocks cut from surgically removed tissue of cervix and vagina of pelvic organ prolapse patients were cultured in extracellular medium (ECM) consisting of RPMI with 15% FCS, 2 mM L-glutamine, 1 mM sodium pyruvate, non-essential amino acids, 100 units/ml penicillin, 100 µg/ml streptomycin, 100 µg/ml gentamicin, and 25 µg/ml amphotericin B. For experiments in the presence of semen or seminal plasma growth medium was supplemented with 100 µg/ml gentamicin to prevent bacterial outgrowth.

Statement

All experiments and methods were performed in accordance with relevant guidelines and regulations. Experiments involving human semen (200/17; 89/16; 351/10), blood and tissues (88/17) were reviewed and approved by Ethics Committee of Ulm University. Informed consent was obtained from all human subjects. All human-derived samples were anonymized before use.

Aminoguanidine treatment of serum and medium

For the treatment of FCS with Aminoguanidine (AG), FCS was supplemented with 5 - 0.5 mM AG and then incubated for 24 hours at room temperature. The AG-treated FCS was then used as a supplement for growth medium. For AG-containing serum-free medium, AG was added at a desired concentration prior addition to cells.

Toxicity assays for cells

Adherent cells were seeded at a density of 2×10^3 cells per well in a flat-bottom 96-well plate in growth medium that was changed the next day to 60 µl of indicated medium for treatment with body fluids and to 90 µl for polyamine treatment. Activated PBMCs were washed, resuspended in indicated medium containing 10 ng/ml interleuckin-2 (IL-2) at a concentration of 1×10^6 cells per ml and then 60 or 90 µl per well were seeded into a 96-well V-bottom plate for body fluids, or polyamine treatment, respectively. Body fluids and compounds were diluted in PBS and 40 µl (semen and seminal plasma), or 10 µl (polyamines) were added to the cells. Viability of adherent cells was determined by a NAD(P)H-dependent colorimetric MTT (3-[4,5-dimethyl-2-thiazolyl]-2,5-dephenyl-2H-tetrazolium bromide) assay according to manufacturer's instructions. Viability of suspension cells was analyzed by CellTiterGlo Luminescence Cell Viability Assay (Promega #G7571) according to the manufacturer's instructions.

Toxicity assay for primary tissues

Vaginal tissue was cut into $2 \times 2 \times 1$ mm^3 blocks as described before.[42,43] 10 blocks per treatment were then treated with 10% or 20% seminal plasma (or buffer) in the presence of chemically defined, serum free medium (X-vivo15) supplemented with 0.005 mM aminoguanidine or in the presence of ECM growth medium supplemented with 10% FCS. After 3 days the individual blocks were washed 2x with PBS and analyzed using CellTiterGlo viability assay.

Statistical analyses

Descriptive data are represented by arithmetic mean and standard error of the mean (SEM). P-values (Fig. 5) are calculated based on linear contrast hypotheses following two-way ANOVA, whereas $p<0.05$ was considered statistically significant. All analyses were conducted in GraphPad Prism (version 9.3.1) and SAS (version 9.4).

Declarations

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Acknowledgements

This work was funded by grants from the German Research Foundation to J.M. (CRC1279 and MU 3115/11-1).

Author contributions statement

M.H. conceived and conducted all experiments except for assay with human tissues, carried out by J-A.M. Human tissues were provided by M.D.. P.v.M confirmed experiments. B.M. performed the statistical analyses. M.H. prepared all figures. J.M. supervised the study and wrote the paper together with M.H. All authors reviewed the manuscript.

Competing Interests

Authors declare no competing interests.

References

1. Haugen, T. B. & Grotmol, T. pH of human semen. Int. J. Androl. **21**, 105–108 (1998).
2. Szczykutowicz, J., Kałuza, A., Kaźmierowska-Niemczuk, M. & Ferens-Sieczkowska, M. The Potential Role of Seminal Plasma in the Fertilization Outcomes. *Biomed Res. Int.* 2019, (2019).
3. Valsa, J., Skandhan, K. P., Sumangala, B. & Jaya, V. Time bound changes (in 24 h) in human sperm motility and level of calcium and magnesium in seminal plasma. Alexandria J. Med. **52**, 235–241 (2016).
4. Gao, J. *et al.* Age-related changes in human conventional semen parameters and sperm chromatin structure assay-defined sperm DNA/chromatin integrity. Reprod. Biomed. Online **42**, 973–982 (2021).
5. Ricci, E. *et al.* Dietary habits and semen parameters: a systematic narrative review. Andrology **6**, 104–116 (2018).
6. Zirafi, O. *et al.* Semen enhances HIV infectivity and impairs the antiviral efficacy of microbicides. Sci. Transl. Med. **6**, (2014).
7. Vallely, P. J. & Rees, R. C. Seminal plasma suppression of human lymphocyte responses in vitro requires the presence of bovine serum factors. Clin. Exp. Immunol. **66**, 181–7 (1986).
8. Vallely, P. J., Sharrard, R. M. & Rees, R. C. The identification of factors in seminal plasma responsible for suppression of natural killer cell activity. Immunology **63**, 451–6 (1988).
9. Szymaniec, S., Quayle, A. J., Hargreave, T. B. & James, K. Human seminal plasma suppresses lymphocyte responses in vitro in serum-free medium. J. Reprod. Immunol. **12**, 191–200 (1987).

10. Kiessling, A. A. Isolation of human immunodeficiency virus type 1 from semen and vaginal fluids. Methods Mol. Biol. **304**, 71–86 (2005).
11. Allen, R. D. & Roberts, T. K. Role of spermine in the cytotoxic effects of seminal plasma. Am. J. Reprod. Immunol. Microbiol. **13**, 4–8 (1987).
12. Roan, N. R. *et al.* Peptides Released by Physiological Cleavage of Semen Coagulum Proteins Form Amyloids that Enhance HIV Infection. Cell Host Microbe **10**, 541 (2011).
13. Kim, K. A. *et al.* Semen-mediated enhancement of HIV infection is donor-dependent and correlates with the levels of SEVI. Retrovirology **7**, 55 (2010).
14. Münch, J. *et al.* Semen-Derived Amyloid Fibrils Drastically Enhance HIV Infection. Cell **131**, 1059–1071 (2007).
15. Rametse, C. L. *et al.* Inflammatory Cytokine Profiles of Semen Influence Cytokine Responses of Cervicovaginal Epithelial Cells. Front. Immunol. **9**, 2721 (2018).
16. Zirafi, O. *et al.* Semen enhances HIV infectivity and impairs the antiviral efficacy of microbicides. Sci. Transl. Med. **6**, (2014).
17. Williamson, J. D. Semen polyamines in AIDS pathogenesis. Nature **310**, 103 (1984).
18. Rodríguez-Páez, L. *et al.* Polyamines Influence Mouse Sperm Channels Activity. Int. J. Mol. Sci. **22**, 1–15 (2021).
19. Pegg, A. E. Toxicity of polyamines and their metabolic products. Chem. Res. Toxicol. **26**, 1782–800 (2013).
20. Jakobsen, H., Rui, H., Thomassen, Y., Hald, T. & Purvis, K. Polyamines and other accessory sex gland secretions in human seminal plasma 8 years after vasectomy. J. Reprod. Fertil. **87**, 39–45 (1989).
21. Shohat, B. *et al.* Immunosuppressive activity and polyamine levels of seminal plasma in azo-ospermic, oligospermic, and normospermic men. Arch. Androl. **24**, 41–50 (1990).
22. Sharmin, S. *et al.* Polyamine cytotoxicity in the presence of bovine serum amine oxidase. Biochem. Biophys. Res. Commun. **282**, 228–235 (2001).
23. Averill-Bates, D. A., Agostinelli, E., Przybytkowski, E. & Mondovi, B. Aldehyde dehydrogenase and cytotoxicity of purified bovine serum amine oxidase and spermine in Chinese hamster ovary cells. Biochem. Cell Biol. **72**, 36–42 (1994).
24. Gahl, W. A. & Pitot, H. C. Reversal by aminoguanidine of the inhibition of proliferation of human fibroblasts by spermidine and spermine. Chem. Biol. Interact. **22**, 91–8 (1978).
25. Stevens, J. F. & Maier, C. S. Acrolein: sources, metabolism, and biomolecular interactions relevant to human health and disease. Mol. Nutr. Food Res. **52**, 7–25 (2008).
26. Parchment, R. E., Lewellyn, A., Swartzendruber, D. & Pierce, G. B. Serum amine oxidase activity contributes to crisis in mouse embryo cell lines. *Proc. Natl. Acad. Sci. U. S. A.* **87**, 4340–4 (1990).
27. Nilsson, B. O. Biological effects of aminoguanidine: an update. Inflamm. Res. **48**, 509–15 (1999).
28. Shore, P. A. & Cohn, V. H. Comparative effects of monoamine oxidase inhibitors on monoamine oxidase and diamine oxidase. Biochem. Pharmacol. **5**, 91–5 (1960).

29. Tamura, H., Horiike, K., Fukuda, H. & Watanabe, T. Kinetic studies on the inhibition mechanism of diamine oxidase from porcine kidney by aminoguanidine. J. Biochem. **105**, 299–306 (1989).
30. Holbert, C. E. *et al.* Autophagy induction by exogenous polyamines is an artifact of bovine serum amine oxidase activity in culture serum. J. Biol. Chem. **295**, 9061–9068 (2020).
31. Wang, L. *et al.* Oxidative degradation of polyamines by serum supplement causes cytotoxicity on cultured cells. Sci. Reports 2018 81 **8**, 1–9 (2018).
32. Wei, X. *et al.* Emergence of resistant human immunodeficiency virus type 1 in patients receiving fusion inhibitor (T-20) monotherapy. Antimicrob. Agents Chemother. **46**, 1896–1905 (2002).
33. Müller, J. A. *et al.* Development of a high-throughput colorimetric Zika virus infection assay. Med. Microbiol. Immunol. **206**, 175–185 (2017).
34. Gilad, G. M. & Gilad, V. H. Polyamines affect growth of cultured rat cerebellar neurons in different sera. Int. J. Dev. Neurosci. **4**, 195–208 (1986).
35. Magnes, C. *et al.* Polyamines in biological samples: rapid and robust quantification by solid-phase extraction online-coupled to liquid chromatography-tandem mass spectrometry. J. Chromatogr. A **1331**, 44–51 (2014).
36. Okamoto, M. *et al.* Seminal plasma induces programmed cell death in cultured peripheral blood mononuclear cells. AIDS Res. Hum. Retroviruses **18**, 797–803 (2002).
37. Selva, K. J., Kent, S. J. & Parsons, M. S. Modulation of innate and adaptive cellular immunity relevant to HIV-1 vaccine design by seminal plasma. AIDS 1 (2016) doi:10.1097/QAD.0000000000001319.
38. Oren, Z., Lerman, J. C., Gudmundsson, G. H., Agerberth, B. & Shai, Y. Structure and organization of the human antimicrobial peptide LL-37 in phospholipid membranes: Relevance to the molecular basis for its non-cell-selective activity. Biochem. J. **341**, 501–513 (1999).
39. Martellini, J. A. *et al.* Cationic polypeptides contribute to the anti-HIV-1 activity of human seminal plasma. FASEB J. **23**, 3609–18 (2009).
40. Bachrach, U. & Rosenkovitch, E. Effect of oxidized spermine and other aldehydes on the infectivity of vaccinia virus. Appl. Microbiol. **23**, 232–5 (1972).
41. Balandya, E., Sheth, S., Sanders, K., Wieland-Alter, W. & Lahey, T. Semen protects CD4 + target cells from HIV infection but promotes the preferential transmission of R5 tropic HIV. J. Immunol. **185**, 7596–7604 (2010).
42. Grivel, J. C. & Margolis, L. Use of human tissue explants to study human infectious agents. Nat. Protoc. **4**, 256 (2009).
43. Müller, J. A. *et al.* Semen inhibits Zika virus infection of cells and tissues from the anogenital region. *Nat. Commun.* 2018 *91* **9**, 1–14 (2018).

Figures

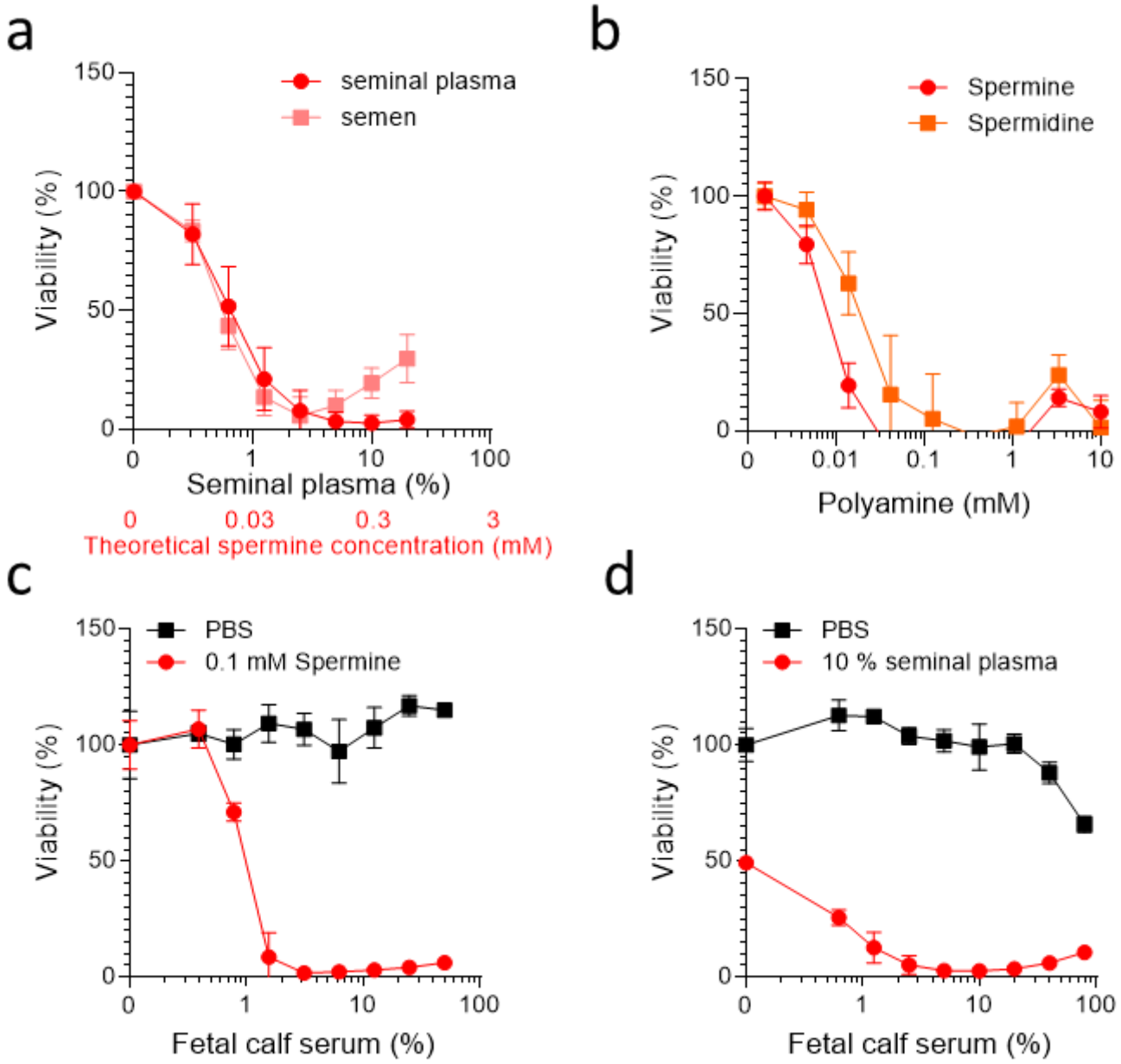

Figure 1

Semen, seminal plasma, spermine, and spermidine are cytotoxic in the presence of FCS. a) TZM-bl cells ($2x10^3$) were treated with serially diluted pooled seminal plasma or semen in the presence of 10% FCS. b) TZM-bl ($2x10^3$) cells were treated with serially diluted synthetic spermine or spermidine in the presence of 10% FCS. c,d) TZM-bl cells ($2x10^3$) were exposed to 0.1 mM spermine or PBS (c), or 10% pooled SP and PBS (d) in the presence of DMEM supplemented with indicated concentrations of FCS. a-d) Viability was determined 2 days later by MTT assay. Data shown are average % values derived from three individual experiments (a,b) or one representative experiment (c,d) performed in biological triplicates ± SD.

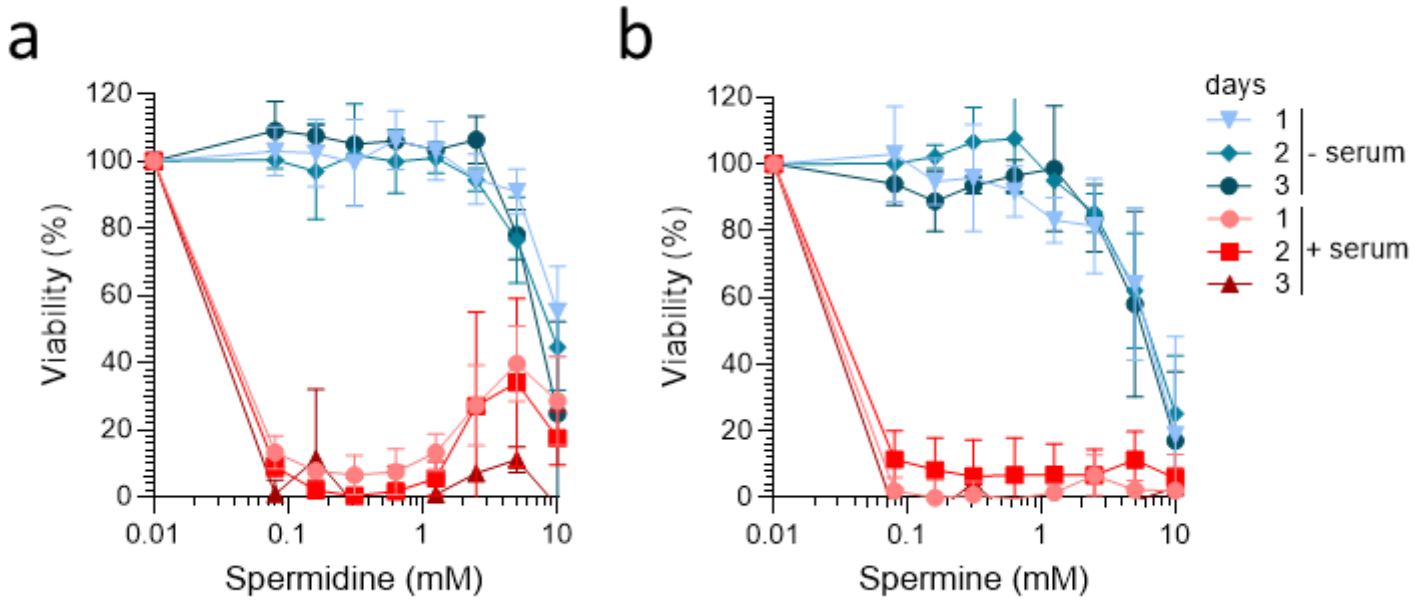

Figure 2

Spermine and spermidine are not cytotoxic under serum-free conditions. TZM-bl cells ($2x10^3$) were incubated with indicated concentrations of a) spermidine and b) spermine in the presence of medium with 10% FCS (+ serum) or chemically defined serum-free medium (- serum). Cell viability was determined after incubation for 1, 2, and 3 days using MTT assay. Data shown are average % values derived from three individual experiments performed in biological triplicates ± SD.

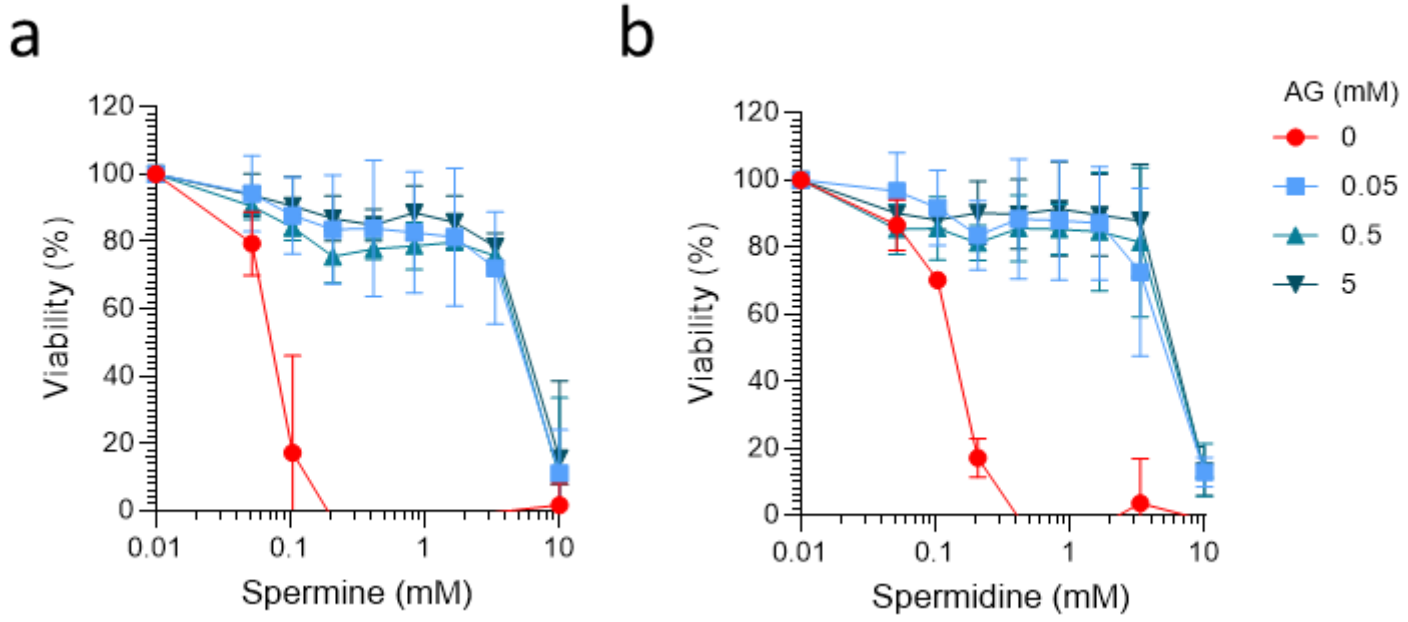

Figure 3

The diamine oxidase inhibitor aminoguanidine prevents FCS-mediated cytotoxic effects of spermine and spermidine. FCS (100%) was preincubated with indicated concentrations of aminoguanidine (AG) for 24 hours. 10-fold dilutions of these samples were then used as cell culture supplement for TZM-bl cells, which were incubated with spermine (a) and spermidine (b) for 2 days. Viability was determined by MTT assay. Shown are average % values derived from 3 individual experiments performed in biological triplicates ± SD.

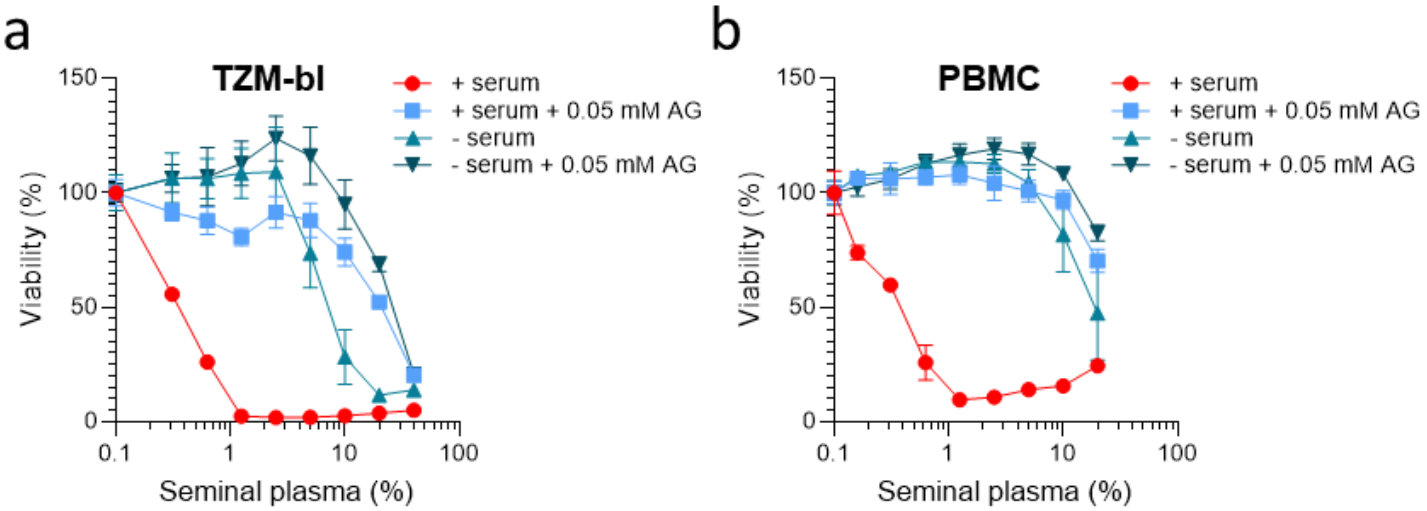

Figure 4

Aminoguanidine combined with serum-free medium prevents seminal plasma-derived cytotoxic effects. a, b) TZM-bl ($2x10^3$) cells (a) or PBMCs ($1x10^5$) (b) were treated with indicated amounts of SP in the presence of growth medium supplemented with 10% FCS, growth medium supplemented with 10% AG-treated FCS (pretreatment with 0.5 mM AG), chemically-defined, serum-free medium, or chemically-defined, serum-free medium supplemented with 0.05 mM AG. Viability was determined after 2 days by MTT assay (a) or 3 days be Cell Titer Glow assay (b). Shown are average % values derived from one representative experiment performed in biological triplicates ± SD.

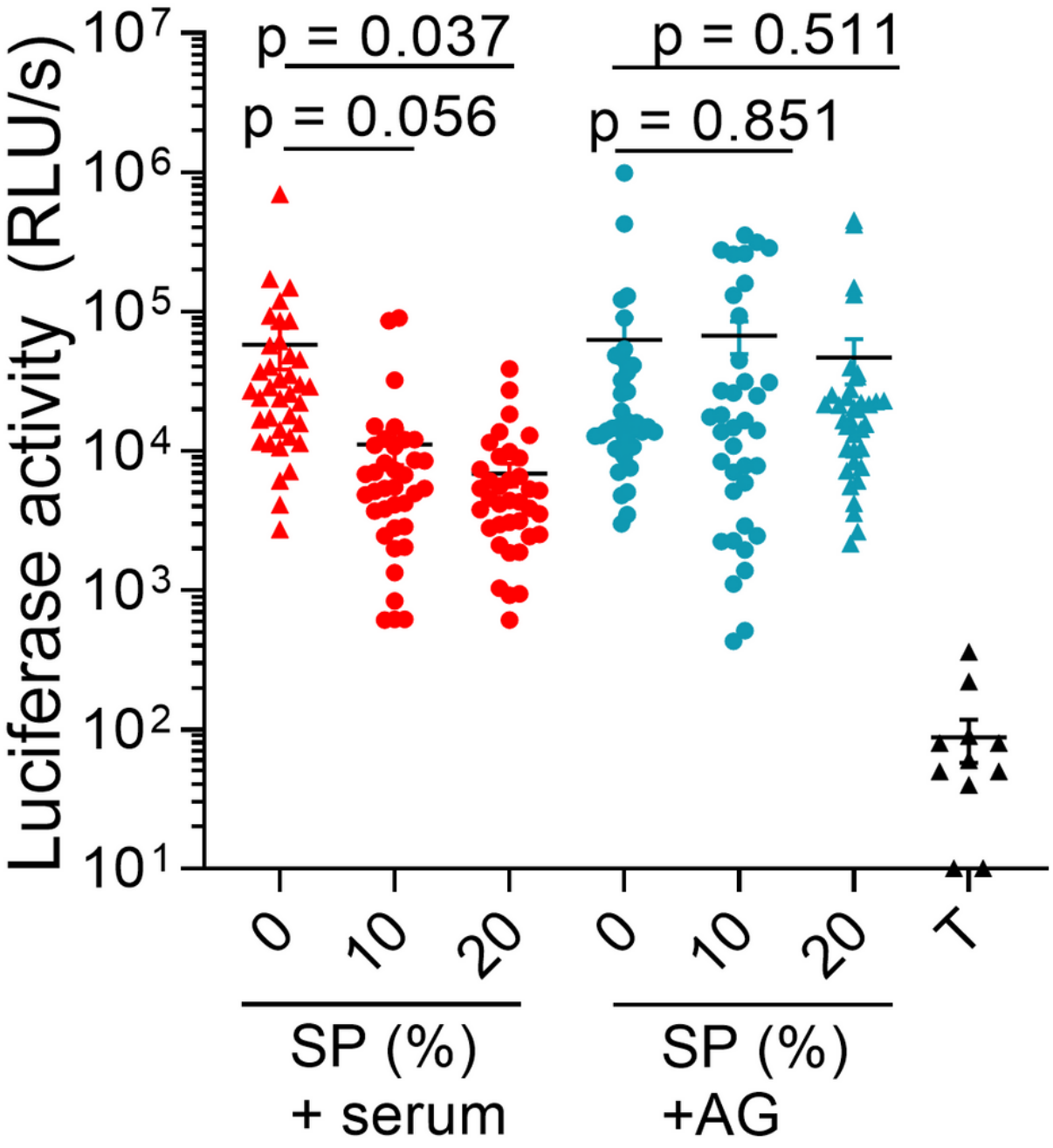

Figure 5

AG prevents SP-induced cytotoxic effects in vaginal tissue blocks. 40 (2 x 2 x 1 mm^3) vaginal tissue blocks derived from 4 individual donors were incubated with buffer, 10% or 20% SP, in the presence of chemically-defined, serum-free medium supplemented with 0.05 mM AG or in the presence of normal growth medium supplemented with 10% FCS. After 3 days the individual blocks were washed twice with PBS and intracellular ATP levels analyzed using CellTiterGlo viability assay. 0.5% triton (T) was used as

positive toxicity control. Black lines indicate arithmetic means ± SEM, RLU/s relative light units per second. P-values are obtained by means of linear contrast hypothesis tests following two-way ANOVA.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementalinformation.docx