Received: 10 October 2019 | Revised: 17 July 2020 | Accepted: 26 August 2020

DOI: 10.1111/and.13859

ORIGINAL ARTICLE

First International Journal of Andrology
aNDROLOGIa
WILEY

Hypotonic challenge reduces human sperm motility through coiling and folding of the tail

Emma Holmes | **Lars Björndahl** | **Ulrik Kvist**

ANOVA, Karolinska University Hospital and Karolinska Institutet, Stockholm, Sweden

Correspondence
Emma Holmes, ANOVA, Karolinska University Hospital and Karolinska Institutet, Norra Stationsgatan 69, S-113 64 Stockholm, Sweden.
Email: emma.holmes@ki.se

Funding information
Nidacon International AB

Abstract

Human ejaculates collected for in vitro procedures show variably rapid increases in osmolality, depending on enzymatic degradation of compounds. Changes in osmolality can affect cell functions due to the energy consuming processes needed to control cell volume. The aim was to examine the effects of a hypotonic challenge for spermatozoa exposed to increased osmolality. Spermatozoa were selected by density gradient centrifugation and washed in media with different osmolalities. Osmolality was measured by freezing-point depression and sperm velocities by CASA. Swimming pattern observations and assessments of tail morphology of fixed spermatozoa were done with phase contrast microscopy. Increased osmolality did not change the curvilinear velocity (VCL), while decreased osmolality reduced or abolished VCL nonreversibly. For spermatozoa first exposed to 400 mOsm/kg, reversal of osmolality to 290 mOsm/kg reduced the VCL and the average path velocity (VAP) permanently. Hypotonic challenges increased sperm tail coiling and folding in a dose-response pattern. Spermatozoa once adjusted to high osmolality in the liquefied ejaculate are likely to suffer if exposed to a medium with a lower osmolality. For improved success of Assisted Reproductive Technologies (ART), it appears to be important to minimise the duration of sperm exposure to the ejaculate, by early dilution or sperm preparation.

KEYWORDS

semen osmolality, sperm motility, sperm preparation, sperm tail coiling, sperm tail folding

1 | INTRODUCTION

During intercourse, spermatozoa leave the isotonic prostate fluid (Holmes et al., 2019b) directly after ejaculation and enter the isotonic cervical mucus (MacLeod & Gold, 1951; Rossato et al., 1996). Therefore, spermatozoa ejaculated during intercourse only mix slightly with the later fractions of the ejaculate dominated by the seminal vesicular fluid. Contrarily, in the laboratory, all ejaculate fractions are, in general, collected and mixed completely. It has been shown that the first fraction of the ejaculate contains both a higher number of spermatozoa and they have better motility than the following fractions (Amelar & Hotchkiss, 1965; Valsa et al., 2012). Collecting ejaculates in split ejaculate fractions is done routinely in several animal species.

When collected in vitro, the human ejaculate undergoes a progressive, enzyme-dependent increase in osmolality (Holmes

et al., 2019a, 2019b). The increment varies between different ejaculates probably due to the variation in relative mixture of glandular secretions in the ejaculate. The rate of increase in osmolality appears to depend primarily on the relative contribution of prostatic fluid to the ejaculate (Holmes et al., 2019b).

Thus, in vitro human spermatozoa are exposed to an increasing osmolality while residing in semen and, cellular mechanisms enable spermatozoa to adjust to the increasing osmolality (Cooper & Yeung, 2003; Petrunkina et al., 2005; Yeung et al., 2006). Following this successive adjustment, spermatozoa prepared for assisted reproduction are exposed to sperm selection media during swim up or density gradient centrifugation. In general, sperm selection media are isotonic to body fluids. The main reason for this appears to be that such media have been developed from cell culture media for somatic cell culture (osmolality in the range 290–300 mOsm/kg). The consequence for spermatozoa adjusted to much higher osmolality would be a risk for a hypotonic challenge when exposed to the sperm selection medium. A hypotonic challenge leads to a momentary water uptake that could be excessive. When spermatozoa are exposed to increasing hypotonicity the tails coil and fold (Drevius, 1963; Kölliker, 1856). Since the sperm membrane surface area does not increase, the cell becomes more spherical and shortens, which forces the tail structure to coil and fold inside the cell membrane (Drevius & Eriksson, 1966). Hypoosmotic swelling also results in lower sperm density and an increased vulnerability to membrane rupture during centrifugation. This is especially true for the initially most swollen spermatozoa (Drevius & Eriksson, 1966). The principle of hypoosmotic swelling is made use of in the HOS-test to distinguish between live, swelling spermatozoa and dead, unaffected spermatozoa (Jeyendran et al., 1992). Furthermore, spermatozoa with coiled tails can be seen in human ejaculates normally (Yeung et al., 2009).

The aim of this study was to investigate how human spermatozoa that have been selected and stored at increased osmolality react when exposed to media that have lower osmolality than that which they were stored at. This will mimic the increase in osmolality that happens during prolonged storage in the semen sample followed by exposure to media.

2 | MATERIAL AND METHODS

2.1 | Recruitment and ejaculate handling

Ejaculates from healthy volunteers were used. All donors gave written consent to the participation (Ethical approval Dnr 2015/2326-31). All samples were collected by the donors, brought to the laboratory and kept at room temperature until completely liquefied. Handling of samples was in compliance with recommendations by the WHO (World Health Organization, 2010) and by the Special Interest Group in Andrology of the European Society of Human Reproduction and Embryology (ESHRE-SIGA) (Barratt et al., 2011).

TABLE 1 Settings for the program Washed Human Semen in Hamilton Thorn IVOS. Average Path Velocity (VAP), Straightness (STR), Straight Line Velocity (VSL)

Image capture	Frames/sec 60Hz	No of frames 30
Cell detection	Min contrast 80	Min cell size 3
Defaults (if <5 motile cells)	Cell size 6 pixels	Cell intensity 160
Progressive cells	VAP (path velocity) 25.0 μm/s	STR (straightness) 80%
Slow cells (included in motile)	VAP cut-off 6.0 μm/s	VSL cut-off 5 μm/s

2.2 | General experimental study design

The aim was to study the effect of hypotonic challenge. To obtain populations of spermatozoa adjusted to specific, stable osmolalities, spermatozoa from donor ejaculates were selected at specific osmolalities by density gradient centrifugation (DGC) and exposed to media with specific osmolalities, thus neutralising the progressive increase in osmolality occurring in untreated ejaculates. Each sample constituted its own control.

2.3 | Motility analysis by computer-assisted sperm analysis (CASA)

CASA was used for the motility assessment of the sperm in media, thus avoiding the difficulties associated with this technique in the assessment of sperm motility in human ejaculates (Mortimer et al., 2015). CASA assessments were done with the Hamilton Thorne (www.hamiltonthorne.com) IVOS version 12.3D (Build 002) on gradient selected and washed spermatozoa using the pre-programmed setting 'washed human'. The detailed settings are presented in Table 1.

The concentration and total number of spermatozoa, motile spermatozoa and progressively motile spermatozoa (≥25 μm/s) were assessed in sperm populations selected by density gradient centrifugation. Also, sperm movement patterns (kinematics: VAP (average path velocity), VCL (curvilinear velocity) and VSL (straight line velocity) were recorded for the sperm populations.

2.4 | Capture of microscope images

Sperm motility was recorded under phase contrast microscopy with the Picsara® Image system (Euromed Networks, www.euromed.se).

2.5 | Preparation of media

Commercially available standard media (provided by Nidacon International AB, www.nidacon.com) were used for washing and

selection of spermatozoa by density gradient, and for extending cell suspensions. The varying osmolalities were achieved by adjusting the glucose, NaCl and KCl levels (Table 2) in the media, and the final osmolality was verified by monitoring measurements. Prior to processing the samples, all the media were brought to room temperature according to manufacturer recommendations. All incubations were performed at 37°C and pH at 7.4–7.8 (HEPES buffered solution) in a temperature-controlled heating cabinet.

2.6 | Measurement of osmolality

The osmolality measurements were made with an Automatic Osmometer (Löser, Svenska Labex AB, www.labex.com) by the principle of freeze point depression as validated and verified earlier (Holmes et al., 2019a).

2.7 | Density gradient centrifugation

For double layer (40/80) density gradients, 1 ml of PureSperm® 80 was added to a conical centrifuge tube. On top of this layer, 1 ml of PureSperm® 40 was carefully layered. Depending on the volume of the ejaculate, a maximum of 750 µl of semen was added on top of the PureSperm® 40 layer. The tube was then centrifuged at 300*g* for 20 min after which the supernatant was removed. The remaining sperm pellet was retrieved and resuspended in 4 ml of washing medium (PureSperm® Wash). This suspension was centrifuged at 500*g* for 10 min after which the supernatant was partially removed to obtain an appropriate sperm concentration (approx. ≤20 million/ml) for subsequent assessment by CASA.

2.8 | Motility after hypertonic and hypotonic challenges

Six different ejaculates were used. After liquefaction and thorough mixing, spermatozoa were selected by density gradient centrifugation. Gradients and washing media were adjusted to 300 mOsm/kg. To obtain the experimental osmolalities, the washed sperm suspensions were split into four aliquots and each aliquot was mixed with washing medium (1 + 3) (PureSperm Wash, Nidacon) with different osmolalities: 300 mOsm/kg, 500 mOsm/kg, 100 mOsm/kg, and 50 mOsm/kg. The mixing schedule and the final osmolalities are shown in Table 3.

Directly after mixing, 5 µl of the sperm suspension was placed in duplicate in a double 20 µm chamber (Leja) and then immediately analysed by CASA. Samples exposed to 150 mOsm/kg ($N = 5$) were

TABLE 2 Preparation of media to obtain different osmolalities in the experiments

Medium type	Osmolalities (mOsm/kg)	Method of preparation
Density gradient	300	Commercially available ready-to-use Nidacon PureSperm® 40 and 80
Density gradient	400	Custom made to the right osmolality. Silanized silica maintained at proportions used in commercial media while other ingredients were increased proportionally to obtain the osmolality of 400 mOsm/kg
Density gradients	290, 310, 330, 350	Commercially available Nidacon PureSperm® 40 and 80 was used and a stock solution with NaCl, KCl and Glucose was used to increase the osmolality to the intended levels. For the 290mOsm/kg gradient, the PureSperm® was diluted with a mix of silanized silica and ddH_2O
Washing medium	50, 100, 290, 300, 310, 330, 350, 400, 500	Commercially available PureSperm® Wash to which a stock solution made with NaCl, KCl and Glucose was added to obtain the intended osmolalities. For the 100 and 50 mOsm/kg, the standard PureSperm® Wash was diluted with ddH_2O and then adding extra hSA to a content of 2.4% to maintain the original protein level
Buffered salt solution	300	Commercially available PureSperm Buffer which is an isotonic HEPES-buffered salt solution which does not contain HSA (human serum albumin)

also assessed 10 min later to evaluate whether any recovery of motility had occurred.

2.9 | Motility after hypotonic challenge following adjustment to high osmolality

In total, 8 different ejaculates were used. Density gradient separation was performed as described, with gradients and washing media adjusted to 400 mOsm/kg. After the last centrifugation, 95 μl of each sample was transferred into each of two Eppendorf tubes, one containing 55 μl of washing medium with osmolality 400 mOsm/kg ("control") and the other containing 55 μl of washing medium with 100 mOsm/kg ("test"; to obtain a final osmolality of 290 mOsm/kg, Table 4). From each of the sperm solutions, 5.5 μl was transferred to a 20 μm Leja chamber and immediately analysed by CASA. The sperm motility in all solutions was analysed directly and after 60 min of incubation at 37°C (CASA settings as in Table 1).

2.10 | Hypotonic challenge and sperm tail coiling and folding

In the series of experiments on motility and hypotonic challenge (as described above) also sperm tail coiling and folding was recorded for aliquots first adjusted to 400 and then exposed to 290 mOsm/kg.

In a second series of experiments, a total of 8 semen samples were used to evaluate effects on sperm tails of more moderate hypotonic challenges. After liquefaction, the samples were carefully mixed and a maximum of 750 μl of each sample was placed on each density gradient. The osmolalities of the density gradient media were adjusted to desired levels: 290 mOsm/kg, 310 mOsm/kg, 330 mOsm/kg and 350 mOsm/kg. After density gradient selection (described above), each selected sperm population was washed (as above) in a washing medium with the same osmolality as the gradient medium. Then, 190 μl of each washed sperm suspension was mixed with a calculated amount of wash medium having an osmolality of 100 mOsm/kg to obtain a final osmolality of 290 mOsm/kg in every test solution. As controls served 190 μl aliquots of each sperm suspension mixed with 100 μl of washing medium with the same osmolality (290, 310, 330 and 350 mOsm/kg respectively) as the original sperm populations after gradient selection and washing, testing different levels of hypotonic challenges (Figure 1). Thus, controls remained in the selection osmolalities while test suspensions were decreased to 290 mOsm/kg. Aliquots selected at 290 mOsm/kg and kept at 290 mOsm served as controls since there was no hypotonic challenge test in these aliquots.

The samples were fixed with a modified Karnovsky solution (2% glutaraldehyde, 2% paraformaldehyde, 0.1 M sodium cacodylate buffer, pH 7.4), after which they were analysed under a phase contrast microscope. Tail appearance (normal, tail tip coiling and tail folding) was classified in four different categories (Figure 2).

2.11 | Statistical methods

Wilcoxon signed-rank test and Pearson's correlation test were performed, using GraphPad Prism version 7.04 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com. $p < .05$ was considered significant.

3 | RESULTS

3.1 | Motility after hypertonic and hypotonic challenges

An increase from the standardised 300 mOsm/kg to 450 mOsm/kg did not affect the motility (Wilcoxon test, VCL; $p = .1563$, n.s., $N = 6$), although a decrease in osmolality to 150 mOsm/kg resulted in a 50% reduced motility (Wilcoxon test, VCL; $p = .0313$, $N = 6$) and a decrease in osmolality to 112.5 mOsm/kg abolished the motility (Wilcoxon test, VCL; $p = .0313$, $N = 6$) (Figure 3). The decrease in VCL after exposure to 150 mOsm/kg did not recover during the 10 min of observation (Wilcoxon test, $p = .8125$; n.s. $N = 5$).

3.2 | Motility after hypotonic challenge following exposure to high osmolality

Exposure of spermatozoa to a medium with an osmolality of 290 mOsm/kg, following an adjustment to 400 mOsm/kg, resulted in a direct, significant reduction in swimming velocity (Wilcoxon test, VCL $p = .0080$; $N = 8$; VAP $p = .0033$; $N = 8$; Figure 4a and 4b) but not in the straight line velocity (Wilcoxon test, VSL; $p = .3125$; $N = 8$; Figure 4c). There was no recovery in proportion of progressive

TABLE 3 Osmolality of sperm preparation after density gradient centrifugation and washing. Osmolality of added medium, volume relation of sperm preparation and added medium, and final osmolalities in experimental sperm suspensions. ($N = 6$)

Osmolality of sperm preparation (mOsm/kg)	Osmolality of added medium (mOsm/kg)	Sperm preparation + medium (relative volumes)	Final osmolality (mOsm/kg)
300	300	1 + 3	300
300	500	1 + 3	450
300	100	1 + 3	150
300	50	1 + 3	112.5

TABLE 4 Composition of sperm solutions to compare motility of spermatozoa first exposed to high osmolality (400 mOsm/kg) and then exposed to osmolality comparable to body fluids (Test: 290 mOsm/kg; Control: 400 mOsm/kg) ($N = 8$)

	Medium		Sperm suspension			
	Volume (μl)	Osmolality (mOsm/kg)	Volume (μl)	Osmolality (mOsm/kg)	Volume final suspension (μl)	Final osmolality of 150 μl (mOsm/kg)
Control	55	400	95	400	150	400
Test	55	100	95	400	150	290

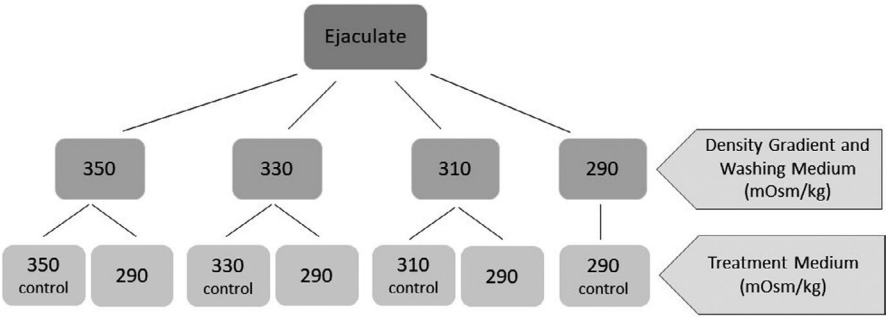

FIGURE 1 Study design for the motility effects of a decrease in osmolality of spermatozoa following an exposure to higher osmolality (350, 330 and 310 mOsm/kg) or kept at 290 mOsm/kg (control). Controls remained in higher osmolality while tests were exposed to 290 mOsm/kg. ($N = 8$)

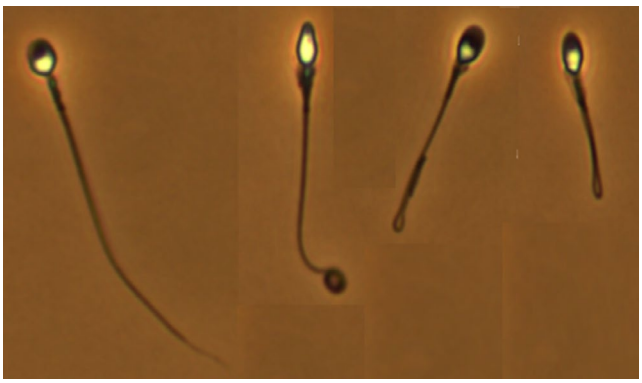

FIGURE 2 Change in appearance of tails upon hypotonic challenge. Grading in four categories (from left to right): 1. Normal: no tail folding or tip coiling; 2. Tip coiling; 3. 50% tail folded; 4, entire tail folded (Photographs by: Emma Holmes)

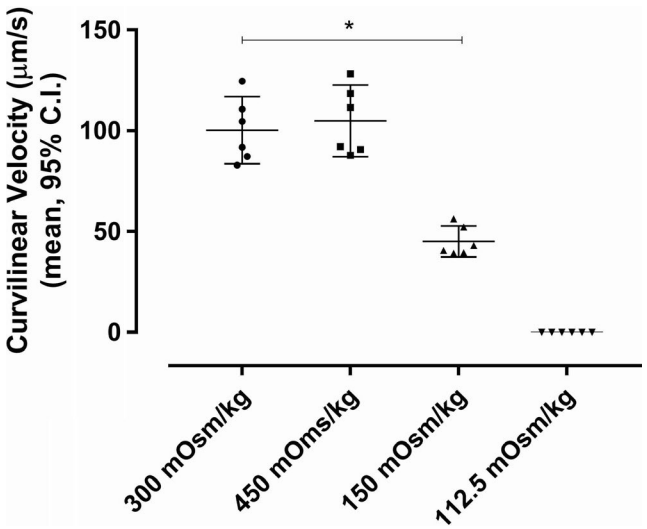

FIGURE 3 Effect of changes in osmolality on sperm motility (Curvilinear velocity VCL) in spermatozoa first exposed to 300 mOsm/kg. Groups are (left to right) spermatozoa exposed to 300 mOsm/kg (controls), 450 mOsm/kg, 150 mOsm/kg and 112.5 mOsm/kg (mean, 95% CI); (cf Table 3). ($N = 6$)

spermatozoa or in swimming velocity (VCL) after 60 min incubation (Wilcoxon test, $p < .0001$; $N = 8$). Also, the proportion of progressive spermatozoa decreased when exposed to lower osmolality (Wilcoxon test, $p = .0031$; $N = 8$; Figure 4d).

3.3 | Hypotonic challenge and sperm tail coiling and folding

A general observation in the first experiments was that tails of spermatozoa exposed to hypotonic changes appeared shorter in the microscope and that the swimming pattern of spermatozoa was altered. Inspection of individual images captured from phase contrast microscopy revealed that the sperm tails had undergone various degrees of tail tip coiling or tail folding. To further characterise the changes in tail appearance, tail morphology of fixed spermatozoa was assessed under phase contrast microscopy and classified in four categories (Figure 2). In a first series of experiments, osmolality was reduced from 400 to 290 mOsm/kg, resulting in a median of sperm tail tip coiling and tail folding of 90% (range 75%–95%) compared with controls (median 8%, range 3%–15%; Wilcoxon test, $p = .0078$, $N = 8$).

In addition, spermatozoa were selected and adjusted to various osmolalities between 310 and 350 mOsm/kg. After exposure to the higher osmolality, spermatozoa were brought back to an environment with 290 mOsm/kg. There was a larger proportion

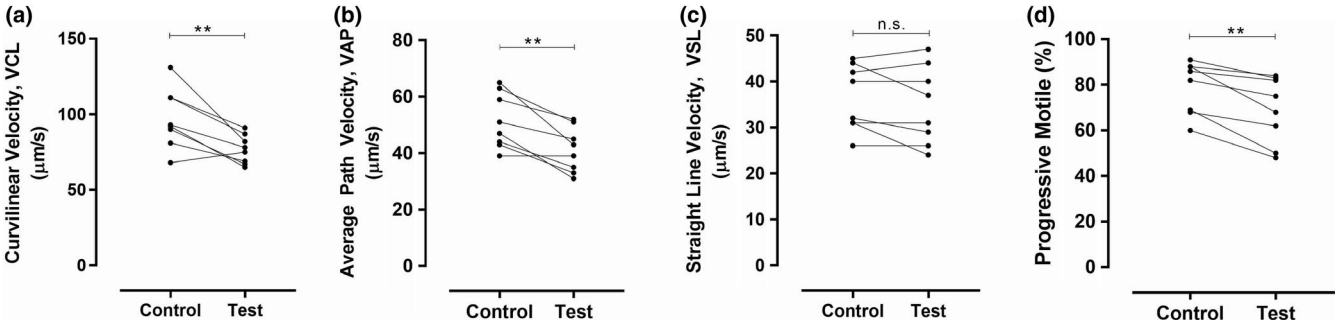

FIGURE 4 Motility after hypotonic challenge (290 mOsm/kg) following exposure and adjustment to 400 mOsm/kg, ($N = 8$): (a) VCL, $p = .0080$. (b) VAP, $p = .0033$. (c) VSL, $p = .3125$. (d) Proportion progressive spermatozoa (VAP ≥ 25 μm/s), $p = .0031$

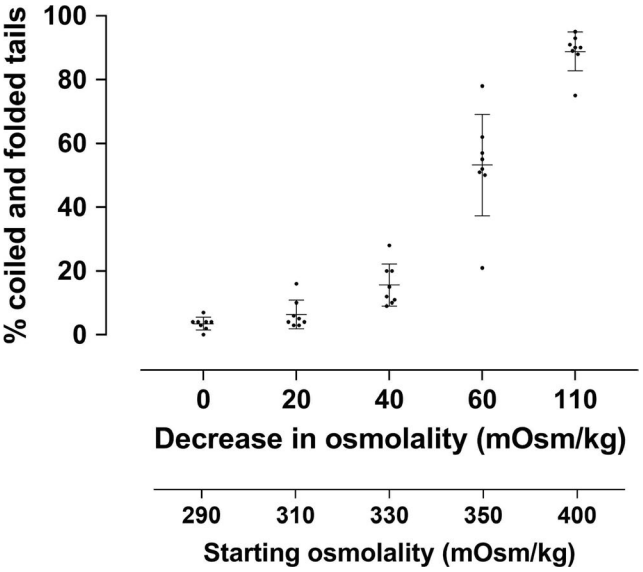

FIGURE 5 Hypotonic challenge and sperm tail coiling and folding: dose-response relation between hypotonic challenge from different starting points (400–310 mOsm/kg) decreased (110–20 mOsm/kg) to 290 mOsm/kg in relation to the proportion spermatozoa with tail coiling and folding. (Pearson correlation test, for each sample ($N = 8$), r-values .92–.98, p-values .0023–.0249)

of spermatozoa with coiled tail tip or folded tail when there was a greater decrease in osmolality (Pearson's correlation test, for each sample ($N = 8$), R-values .92–.98, p-values .0023–.0249), showing a dose-response relation (Figure 5).

4 | DISCUSSION

In this experimental study, it was hypothesised that, since the osmolality of semen increases after ejaculation by varying degree depending both on time and individual samples (Holmes et al., 2019a, 2019b), when the sample then is exposed to a selection medium with lower osmolality the sperm cells will take up water and swell. This in turn will affect morphology and motility.

An increase in osmolality (hypertonic challenge) from 300 to 450 mOsm/kg did not affect sperm motility (Figure 3) although prolonged exposure to even higher osmolality can cause a decreased motility (Makler et al., 1981). In contrast, decreased osmolality (hypotonic challenge) from 300 mOsm/kg decreased sperm motility (Figure 3) without recovery within 10 min. This is in congruence with earlier reports of decreased sperm motility due to a hypotonic challenge (Drevius & Eriksson, 1966; Kölliker, 1856; Lindahl & Drevius, 1964; Makler et al., 1981).

In this study, both sperm velocity (VCL, VAP) and the proportion of progressive spermatozoa decreased upon a hypotonic challenge, and no recovery was observed during one hour of observation. It can be concluded that motility is negatively affected by a hypoosmotic challenge followed by treatment in isotonic media and the motility does not recover. This is in accordance with the observation that neither tail appearance nor sperm motility normalised spontaneously after a hypertonic challenge (Drevius & Eriksson, 1966). In addition, the motility changed to a less curvilinear pattern, since VCL and VAP decreased whereas the VSL did not change. Furthermore, that most spermatozoa exposed to a hypotonic challenge revealed changes in tail appearance (tail tip coiling and tail folding, Figure 2, Figure 5) indicates that tail coiling and folding can be the reason for the decrease in overall motility and change in motility patterns.

Even lesser hypotonic challenges, similar to what can occur in routine laboratory practice (Holmes et al., 2019a) can affect sperm tail appearance and motility. In fact, there appeared to be dose-response effect showing a significant increase of sperm tail-tip coiling and tail folding for spermatozoa first adjusted to an osmolality above 330 mOsm/kg and then exposed to isotonic osmolality (Figure 5).

The hypotonic challenges are likely to be of importance for sperm selection in vitro. Earlier studies have indicated that osmolality above 330 mOsm/kg can be present in many semen samples left for 60 min before preparation of spermatozoa (Holmes et al., 2019a; Makler et al., 1981; Yeung et al., 2009). Thus, in addition to pH and temperature osmolality appears to be a crucial factor to control in order to preserve normal sperm functionality. However, routine measurements of semen osmolality would only reveal a progressive increase in osmolality with time after ejaculation (Holmes et al., 2019a). Such measurements are thus less relevant than minimising sperm exposure to the development of high osmolality in the ejaculate. One way to accomplish this would be to reduce the time in semen before sperm preparation (Holmes et al., 2019a). Other possibilities could

be early dilution after ejaculation (Holmes et al., 2019b) or use of the first, sperm-rich ejaculate fraction (Amelar & Hotchkiss, 1965; Björndahl & Kvist, 2003; Holmes et al., 2019a).

ACKNOWLEDGEMENT

Commercially available standard media were provided by Nidacon International AB, www.nidacon.com. Ph.D. grant for Emma Holmes was provided by Nidacon International AB.

DATA AVAILABILITY STATEMENT

Data from this experimental, in addition of what is available in the published material, can be obtained from the authors.

ORCID

Emma Holmes https://orcid.org/0000-0002-6619-515X

Lars Björndahl https://orcid.org/0000-0002-4709-5807

REFERENCES

Amelar, R. D., & Hotchkiss, R. S. (1965). The split ejaculate: Its use in the management of male infertility. *Fertility and Sterility*, *16*, 46–60. https://doi.org/10.1016/S0015-0282(16)35466-8

Barratt, C. L., Björndahl, L., Menkveld, R., & Mortimer, D. (2011). ESHRE special interest group for andrology basic semen analysis course: A continued focus on accuracy, quality, efficiency and clinical relevance. *Human Reproduction*, *26*(12), 3207–3212. https://doi.org/10.1093/humrep/der312

Björndahl, L., & Kvist, U. (2003). Sequence of ejaculation affects the spermatozoon as a carrier and its message. *Reproductive BioMedicine Online*, *7*(4), 440–448. https://doi.org/10.1016/S1472-6483(10)61888-3

Cooper, T. G., & Yeung, C. H. (2003). Acquisition of volume regulatory response of sperm upon maturation in the epididymis and the role of the cytoplasmic droplet. *Microscopy Research and Technique*, *61*(1), 28–38. https://doi.org/10.1002/jemt.10314

Drevius, L. O. (1963). Spiralization in tails of mammalian spermatozoa in hypotonic media. *Nature*, *197*(4872), 1123–1124. https://doi.org/10.1038/1971123b0

Drevius, L. O., & Eriksson, H. (1966). Osmotic swelling of mammalian spermatozoa. *Experimental Cell Research*, *42*(1), 136–156. https://doi.org/10.1016/0014-4827(66)90327-2

Holmes, E., Björndahl, L., & Kvist, U. (2019a). Post-ejaculatory increase in human semen osmolality in vitro. *Andrologia*, *51*, e13311. https://doi.org/10.1111/and.13311

Holmes, E., Björndahl, L., & Kvist, U. (2019b). Possible factors influencing post-ejaculatory changes of the osmolality of human semen in vitro. *Andrologia*, *51*, e13443, https://doi.org/10.1111/and.13443

Jeyendran, R. S., Van der Ven, H. H., & Zaneveld, L. J. (1992). The hypoosmotic swelling test: An update. *Archives of Andrology*, *29*(2), 105–116. https://doi.org/10.3109/01485019208987714

Kölliker, A. (1856). *Physiologische Studien über die Samenflussigkeit* (Vol. VII, pp. 201–272). Zeitschrift für Wissenschaftliche Zoologie.

Lindahl, P. E., & Drevius, L. O. (1964). Observations on bull spermatozoa in a hypotonic medium related to sperm mobility mechanisms. *Experimental Cell Research*, *36*, 632–646. https://doi.org/10.1016/0014-4827(64)90319-2

MacLeod, J., & Gold, R. Z. (1951). The male factor in fertility and infertility. III. An analysis of motile activity in the spermatozoa of 1000 fertile men and 1000 men in infertile marriage. *Fertility and Sterility*, *2*(3), 187–204. https://doi.org/10.1016/S0015-0282(16)30540-4

Makler, A., David, R., Blumenfeld, Z., & Better, O. S. (1981). Factors affecting sperm motility. VII. Sperm viability as affected by change of pH and osmolarity of semen and urine specimens. *Fertility and Sterility*, *36*(4), 507–511. https://doi.org/10.1016/S0015-0282(16)45802-4

Mortimer, S. T., van der Horst, G., & Mortimer, D. (2015). The future of computer-aided sperm analysis. *Asian Journal of Andrology*, *17*(4), 545–553. https://doi.org/10.4103/1008-682X.154312

Petrunkina, A. M., Jebe, E., & Topfer-Petersen, E. (2005). Regulatory and necrotic volume increase in boar spermatozoa. *Journal of Cellular Physiology*, *204*(2), 508–521. https://doi.org/10.1002/jcp.20317

Rossato, M., Di Virgilio, F., & Foresta, C. (1996). Involvement of osmo-sensitive calcium influx in human sperm activation. *Molecular Human Reproduction*, *2*(12), 903–909. https://doi.org/10.1093/molehr/2.12.903

Valsa, J., Skandhan, K. P., Khan, P. S., Sumangala, B., & Gondalia, M. (2012). Split ejaculation study: Semen parameters and calcium and magnesium in seminal plasma. *Central European Journal of Urology*, *65*(4), 216–218. https://doi.org/10.5173/ceju.2012.04.art7

World Health Organization. (2010). *WHO laboratory manual for the examination and processing of human semen*, 5th ed. World Health Organization.

Yeung, C. H., Barfield, J. P., & Cooper, T. G. (2006). Physiological volume regulation by spermatozoa. *Molecular and Cellular Endocrinology*, *250*(1–2), 98–105. https://doi.org/10.1016/j.mce.2005.12.030

Yeung, C. H., Tuttelmann, F., Bergmann, M., Nordhoff, V., Vorona, E., & Cooper, T. G. (2009). Coiled sperm from infertile patients: Characteristics, associated factors and biological implication. *Human Reproduction*, *24*(6), 1288–1295. https://doi.org/10.1093/humrep/dep017

How to cite this article: Holmes E, Björndahl L, Kvist U. Hypotonic challenge reduces human sperm motility through coiling and folding of the tail. *Andrologia*. 2020;52:e13859. https://doi.org/10.1111/and.13859