Nidacon News

The news letter from your ART supplier • No 2 • 2020

There are three good rules to apply in this life "Learn from yesterday, live today and plan for tomorrow".

Suddenly we found ourselves in the middle of a pandemic and the effects became detrimental to many of us.

At Nidacon we quickly realized that all of us except those absolutely necessary had to work from home. We changed routines to make it safe to be at the We had meetings where we discussed how we could contribute to this new situation and we contacted the purchasing centrals and offered to be middle hand to sources of needed materials. We early decided that although many of our customers were forced to close down, we had to keep open for those that did not. We kept communicating, producing and

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mers from now on thanks to the virtual format now being globally implemented. Maybe we will travel less with flights, maybe we will not take the car to work every day, since we can work from home and in that case maybe this will continue being a relief from our carbondioxide trail.



office but all that could, should work from home. We as so many other businesses found ourselves having several months with a third of our normal sales. We immediately slimmed down all costs.

We found ourselves actually having all the systems necessary for being able to have meetings virtually. All flights were cancelled, and the IVF-conferences changed into virtual format. This delivering throughout the worst months.

We are still in the middle of the pandemic and maybe it will never fully disappear. Maybe we will just learn how to live with it. In Sweden for

example we no longer greet people with taking their hands we have found alternative methods.

We believe that our team and actually all of us together have a chance to learn from

this rough spot and build something new and maybe better for the future. Maybe we can meet and have more chat communications and meetings with our custoWe can be found on Facebook, LinkedIn and Instagram and we have active communications on WhatsApp, WeChat and Line. In all of them we are available to chat with. We utilize Skype, telephone conferences, Teams and Zoom for our meetings.

We hope to meet you in these new formats and in this new and maybe improved world where we all are fast at adjusting and taking care of each other and our surroundings.



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We found ourselves actually having all the systems necessary for being able to have meetings virtually.

worked fine. It is not the same as a physical meeting, but we can at least see the customers and chat and get the so important feedback.

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

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INTRODUCTION

During intercourse, spermatozoa leave the isotonic prostate fluid (Holmes et al., 2019b) directly after ejaculation and enter the isotonic cervical mucus (Mac-Leod & 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.

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 HOStest 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.

RESULT AND 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).



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)



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)

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.

The hypotonic challenges are likely to be of importance for sperm selection in vitro. Earlier studies have indicated that



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)

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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).

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This article is a part of Emma Holmes thesis for her Ph.D "On osmolality and sperm function during processing for assisted reproduction".

If you have any questions or like to know more, please do not hesitate to contact us.



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Nidacon News

Customer feedback

Customer feedback and collaboration is essential to guide our decision making and influence innovations and changes.

We like you to know what a difference you can make with only a small comment regarding a product or an instruction for use.

We have through the years after collaboration and feedback with customers released new products, updated, and refined instructions for use and improved our services.

VitriBlast and ThermoBlast was developed together with LIVIO Göteborg, several instructions for use have been



updated with the help of our customers, packaging have changed with the help of your ideas and thoughts.

So, if you have ideas, small changes that you have made to our instructions or maybe tests you would like to perform using our products, let us know. In December we will send out our customer survey and we would really appreciate if you can find the time to share your opinions.

Thank you and stay safe.



> Who to contact

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