Nidacon News

The news letter from your ART supplier • No 1 • 2020

Contents

Emma Holmes's dissertation

On April 15, 2020, it was time for Emma Holmes to defend her thesis "On Osmolality and Sperm Function During Processing for Assisted Reproduction".

Emma during the examination part.

Emma started working at Nidacon in 2005 after finishing her undergraduate degree in Science at the University of Guelph, Canada. During her Ph.D. studies she has been working solely in Research and Development at Nidacon meanwhile she has previously also worked in Marketing as a Product Specialist. She regularly gives presentations at our workshops and also visits clinics to talk about her research.

The dissertation was held at ANOVA, part of the Karolinska Hospital, Stockholm. The opponent was Dr. Jackson Kirkman-Brown (University of Birmingham), and the examination board included Dr. Kersti Lundin (University of Gothenburg), Dr. Heriberto Rodriguez-Martinez (University of Linköping) and Dr. Moustapha Hassan (Karolinska Institute).

Due to the prevailing situation with travel restrictions etc. due to the corona pandemic, most people could not attend, instead the entire defense was held on Zoom with a just a few people still attending in person. Invitations had been sent out in advance and the online set-up was very well organized and worked perfectly without any complications or interruptions. In comparison to a normal dissertation this way people from all over could attend which was very positive! The main supervisor namely Dr. Ulrik Kvist was attending from his home just outside of Stockholm.

The presentation itself lasted for about 1 hour followed by intriguing questions from the opponent with the answers often leading to an interesting discussion. It was evident that the opponent has a genuine interest in the subject of Andrology and the current methods used to analyze, handle and treat spermatozoa in the ART laboratory. His extensive knowledge and experience in the field was also apparent. The examination board also had some very interesting questions and with them coming from a wide range of fields of expertise they covered the thesis very nicely. While the examination board had their meeting after the examination the rest of the group had some time to enjoy some snacks while waiting for the official result.

The official result was in and the dissertation was accepted, it passed!! Time for a well-deserved cheer! From the left, Dr. Lars Björndahl (co-supervisor), Emma Holmes (new Ph.D.), Dr. Stefan Arver (head of the dissertation).

Photos: Magdalena Larsson Chatziantonis, Lars Björndahl.

Unfortunately, we greatly missed the main supervisor Dr. Ulrik Kvist at this gathering. We will have to celebrate in person at a later date. We also greatly missed the other co-supervisor Dr. Peter Sjöblom who sadly passed away in 2012. Overall, it was a very successful dissertation and thankfully the technology worked perfectly and finally the result was positive! Now starts the hard work with continued testing and making sure this work will make a difference.

Cheers and thank you to everyone that could attend!

THESIS ABSTRACT

Deep basic knowledge about sperm physiology is relevant and important to optimize the outcome of procedures used during Assisted Reproductive Technologies (ART) to select spermatozoa for fertilization.

More specifically, this study examined osmolality changes and consequences for sperm motility and sperm selection in the laboratory. What kind of environmental changes occur and what challenges must the spermatozoa endure after leaving the body? How do these challenges affect the spermatozoa's functions, fertilizing potential and the make-up of the genetic material they will deliver to the oocyte?

In study I, the objective was to measure the changes in osmolality that occur after collecting the ejaculate in the laboratory. After ejaculation, the sample is mixed in order to make it homogenous. This will cause the different fractions that make up the semen sample to mix. A total of 348 individual ejaculates, 5 split ejaculates and 6 ejaculate pools were used in all 4 studies. It appeared that there was an individual pattern of change in osmolality over time. At 3 hours after the ejaculation, the change in osmolality ranged from 2 mOsm/kg to 164 mOsm/kg.

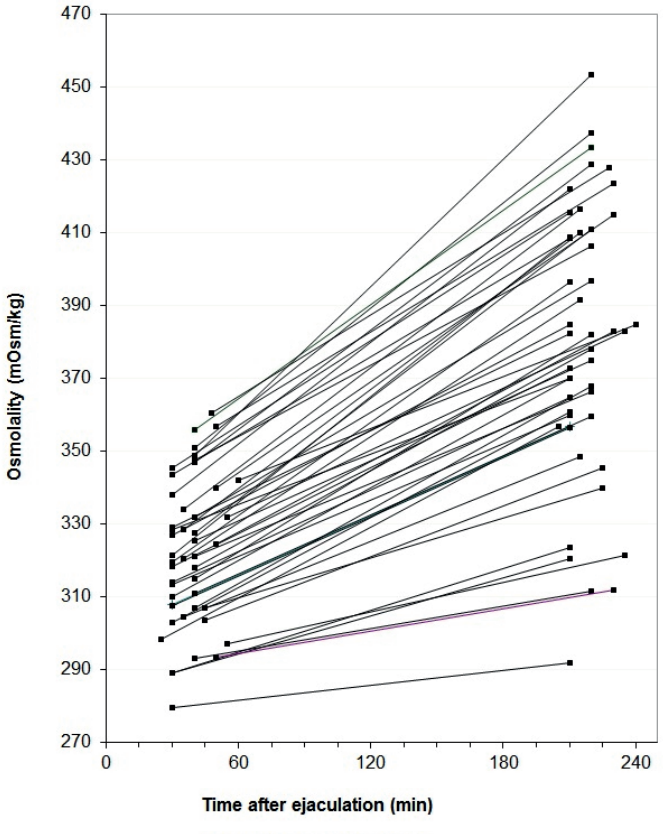

Figure 1. Osmolality during 3 hours of storage in 47 individual ejaculates.

Furthermore, it was evident that the change in osmolality was temperature dependent. Samples stored at 37°C increased significantly more in osmolality than samples stored at 18-22°C, than samples stored at 4-7°C and, than samples stored at -20°C.

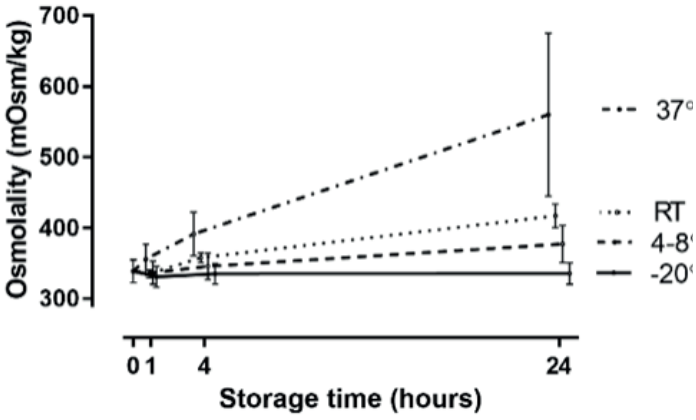

Figure 2. Development of osmolality in ejaculates during storage in different temperatures.

Denaturing temperature (100°C) blocked any further increment in osmolality. One probable cause of the increase in osmolality is that the enzymes, which are abundant in the prostatic fluid, are degrading macro-molecules, such as the proteins that are abundant in the seminal vesicular fluid. When these two secretions are mixed, the enzymatic degradation can start (Mann and Lutwak–Mann, 1981).

In study II, the markers for the different fractions of the ejaculate were measured in order to relate to the change in osmolality. As well as containing high levels of proteins, the seminal vesicular fluid also contains relatively high levels of fructose. Similarly, the prostatic fluid contains high levels of zinc. It was shown that 19% of the variation in semen osmolality covaried with the relative contribution of the prostatic fluid marker, zinc, while the seminal vesicular marker, fructose and the epididymal marker neutral α-glucosidase did not covary.

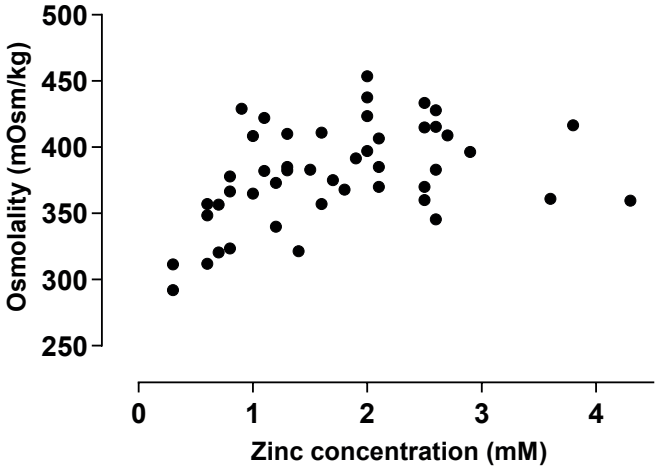

Figure 3. Correlation between change in osmolality over time and concentration of prostatic marker, zinc.

Furthermore, the results show that after removing sperm from the ejaculate, the osmolality still increased, thus, the sperm did not have any effect on the increase.

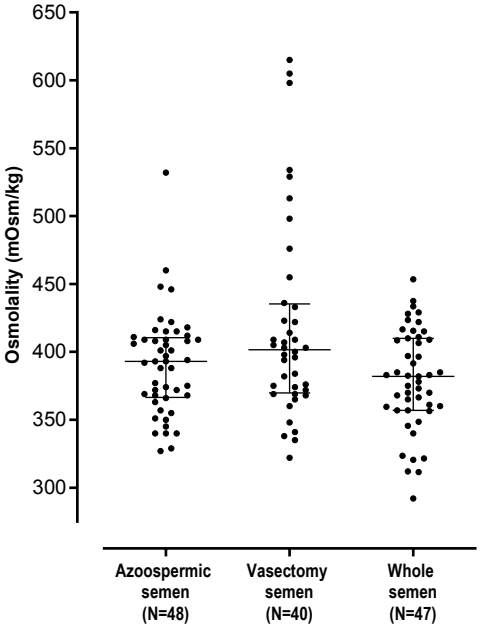

Figure 4. Comparison of development in osmolality between azoospermic semen, vasectomy semen and whole semen.

In addition to the challenge of the osmotic increase occurring in the ejaculate, the preparation of the sperm for ART presents yet another challenge. Most commercial sperm preparation media, such as density gradients or swim-up media have an adjusted osmolality of 290-300mosm/kg (isotonic to body fluids). Thus, depending on the individual increase in osmolality of the samples, the sperm will be exposed to varying sudden decreases in osmolality during preparation. The increment in osmolality can be reduced by diluting the samples after ejaculation

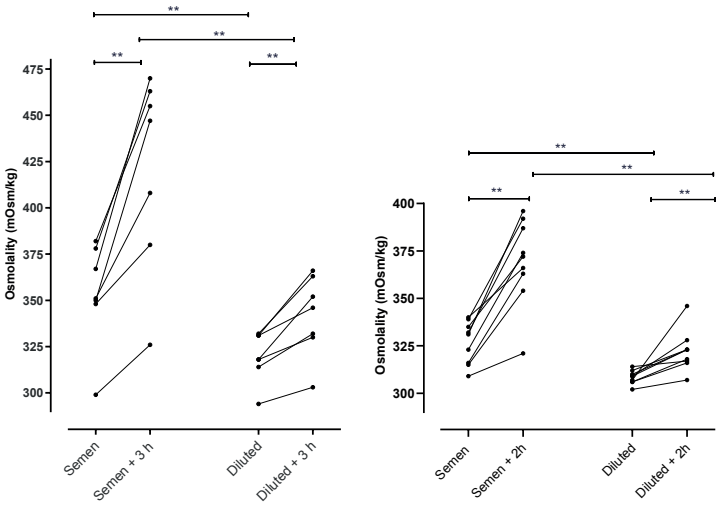

Figure 5. The increment in osmolality can be reduced by diluting the sample shortly after ejaculation. Here is the development in osmolality in diluted ejaculates compared to non-diluted (semen) during 3 hours at 37°C (left) and at room temperature (right).

In study III and IV, it was examined how a hypo-osmotic challenge could affect sperm motility and the outcome of sperm selection when using density gradient centrifugation. Sperm motility was assessed by Computer Assisted Sperm Analysis (CASA). When the spermatozoon was exposed to a sudden decrease in osmolality, it took up water and swelled, causing the tail to coil and fold.

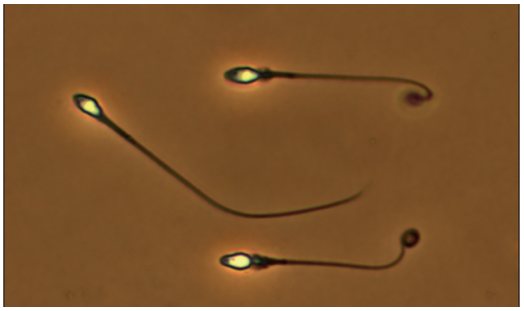

Figure 6. Picture of spermatozoa exposed to a drop in osmolality from 400 mOsm/kg to 290 mOsm/kg. Sperm tail coiling is apparent.

This in turn, resulted in a decreased motility (VCL) with as much as 20%.

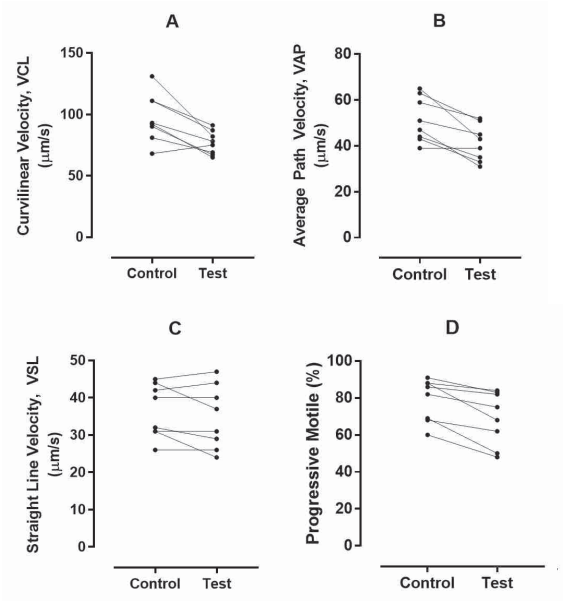

Figure 7. Motility after hypotonic challenge, that is a drop from 400 mOsm/kg to 290 mOsm/kg. Curvilinear Velocity (VCL) is reduced as well as Average Path Velocity (VAP) and Progressively Motile (PM), meanwhile Straight Line Velocity remains the same.

Furthermore, it appears that the greater the decrease in osmolality, the lower the yield was after selection of spermatozoa by density gradient centrifugation.

In contrast, with further investigation, it was shown that the DNA-Fragmentation-Index (DFI), measured by flow cytometry of acridine-orange stained spermatozoa was not affected by longer incubation times. However, spermatozoa ejaculated directly into a buffer had lower values for DFI% compared to samples diluted with buffer shortly after ejaculation.

The negative effect on the yield was eliminated when the ejaculate was diluted soon after ejaculation or collected directly in a buffered solution.

Since the increase in osmolality in vitro is so variable, one standardized procedure for sperm preparation would not work for all ejaculates. However, if increasing osmolality can be minimized by early dilution of all samples, then the negative effects can in large be eliminated.

Product development
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Nidacon News No 1 • 2020

Upcoming events

- **ESHRE 36th Virtual Annual meeting** 5-8 July, 2020.

European Society of Human Reproduction and Embryology

- **10th Congress of the Asia Pacific Initiative on Reproduction (ASPIRE 2020)**
  4–7 August, 2020.
  Manila, Philippines.

- **Swedish Society for Reproductive Medicine Annual meeting**
  20-22 August, 2020. Umeå, Sweden.

SSRM
Svenska Sällskapet för Reproduktionsmedicin
Swedish Society for Reproductive Medicine

- **The Nordic IVF-Laboratory Society (NILS)**
  25-26 September, 2020. Helsinki, Finland.

NILS
Nordic IVF Laboratory Society

- **ASRM Scientific Congress**
  17-21 October, 2020. Portland, USA.

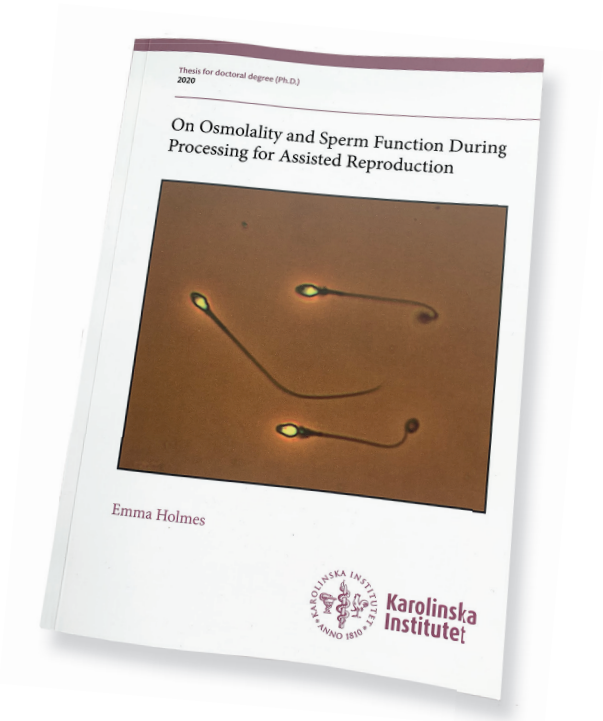
AMERICAN SOCIETY FOR REPRODUCTIVE MEDICINE
ASRM 2020
SCIENTIFIC CONGRESS & EXPO
PORTLAND, OREGON OCTOBER 17-21, 2020

If you are interested in reading Emma Holmes published thesis, you can use this link

https://news.ki.se/dissertation-emma-holmes-anova?_ga=2.253476746.2031069908.1586240133-1002348444.1552062989

We have some paper copies, please let us know if you would like us to send you one.

You can of course contact Emma if you have any questions or comments regarding her thesis
emma@nidacon.com



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