INVESTIGATION OF OPERATING PARAMETERS FOR A SEMEN QUALITY ANALYSIS SYSTEM

S. Atherton, C. R. Evans, P. Roach, D. C. Hughes, G. McHale and M. I. Newton
School of Science and Technology
Nottingham Trent University, Clifton Lane, Nottingham
NG11 8NS, U.K.

Keywords: Sperm, Semen, Motility, Acoustic wave, QCM, Time of flight.

Abstract: To increase the success rate of Artificial Insemination (AI) in animals, it is important that the semen sample is of a high quality. The quality is related to both the number and motility of sperm present. Numerous methods of analysing semen samples exist, but these are generally expensive and/or laboratory based. A useful alternative would be an inexpensive simple system that could be used in the field immediately prior to insemination. We present a time of flight (ToF) technique using a quartz crystal microbalance (QCM). In this system the sperm are introduced at one end of a liquid filled swim channel and self propel to a QCM sensor at the other end. A chemical coating is applied to the QCM to bind the sperm and from the frequency change the number of attached sperm and their ToF can be measured. We report the effect of temperature and the introduction of small quantities of progesterone into the swim channel on the sperm ToF. Results show the QCM can be used to detect the arrival of the sperm and that increasing temperature and the presence of progesterone are both shown to decrease the ToF.

1 INTRODUCTION

Within the Artificial Insemination (AI) industry it is important to be able to measure the concentration of viable sperm in a sample. AI is a common procedure in farm animals, more than 100 million inseminations are performed globally every year. It is not only the number of sperm in a semen sample that is crucial to the success of the insemination process, but also the motility of the sperm. Whilst having a large number of motile sperm in a sample is not a guarantee of fertility, it is an excellent indicator of semen quality.

Two optical methods of performing sperm counts are the haemocytometer and counting chambers. The drawback of these methods is that multiple measurements are needed to achieve an acceptable level of precision, resulting in a more time consuming procedure. The reason for the relative inaccuracy of these methods is due to the rapid movement of the sperm under high magnification and the tedious nature of the work for the human operator. It is possible to perform a more objective assessment of sperm motility using a computer assisted semen analyzer (Mortimer, 2000), which is a laboratory based instrument that can measure different aspects of the sperm movement. To further increase precision, a combination of fluorescent staining and flow cytometry can be used to analyze thousands of sperm in a sample (Christensen et al, 2005). The common drawback with all of the above techniques is the price of the equipment itself and the need for a skilled operator.

In some species the sample may be successfully frozen and thawed before use however this is not always the case. The sperm of other species cannot be successfully frozen and so a fresh sample, with a shelf life of only a few days, must be used. Increasingly, AI is being used for equine applications and recent changes in UK legislation (Artificial Insemination of Mares Order 2004) mean that lay (non-Veterinary) and farm workers are now being trained to perform such inseminations. For sport equine applications the cost of semen for a single mare to be covered may exceed £1000. Semen analysis is currently a specialist, subjective and skilled process that is normally carried out under laboratory conditions. Given these trends, a low cost, simple to use and objective technique to assess the quality of the semen, particularly under field conditions just before insemination, would greatly

Atherton S., R. Evans C., Roach P., C. Hughes D., McHale G. and I. Newton M. (2009).
INVESTIGATION OF OPERATING PARAMETERS FOR A SEMEN QUALITY ANALYSIS SYSTEM.
In *Proceedings of the International Conference on Biomedical Electronics and Devices*, pages 13-16
DOI: 10.5220/0001429600130016

improve the quality and practice of artificial insemination in animals. It would also provide an easier and more cost effective method for monitoring male animal fertility and breeding male welfare.

Acoustic wave sensors detect very small changes in mass attached to their surface and often contain a sensitizing layer that can recognize and bind the species to be detected onto the mass sensitive surface. The quartz crystal microbalance (QCM) is the most widely used acoustic wave device for sensor applications.

$$\Delta f = -2.26 \times 10^{-6} f^2 \Delta m/A \quad (1)$$

The Sauerbrey equation (Sauerbrey, 1959) relates the change of the crystals resonant frequency to the change in rigid mass on the crystal surface; this is shown for AT cut quartz in equation 1 where Δf (in Hz) is the change in frequency that occurs for an increase in mass Δm (in grams) on the surface of area A (in cm^2) with a crystal resonant frequency of f (in Hz) and the constant comes from the crystal materials properties. A well-designed oscillator circuit can still resonate a crystal even under the high damping caused by immersion in a liquid. The change in mass rigidly attached to the surface still causes a proportional change in frequency although changes in other parameters such as the liquids viscosity and density will also cause changes in frequency. The acoustic wave will only sense mass changes within a short distance into the liquid called the penetration depth. (Kanazawa & Gordon, 1985) Previous studies have shown up to 70% of the sperm mass to be made up of water (Da Silva et al, 1992) so it is not obvious how the attachment of a sperm will change the QCM response. In a preliminary report (Newton et al, 2007) we have fitted the resonance curves of 5MHz QCM to the Butterworth van Dyke model and this has shown that the sperm may be treated a rigid mass and so a model based on the Sauerbrey equation is appropriate when using an *effective mass* of around 5pg. For other operating frequencies or other species sperm this effective mass would be different.

In this report we extend this preliminary work to investigate the effect of environmental parameters on the time of flight (ToF). For any practical measurement technique it is essential the time the measurement takes is sufficiently short to be usable. For a portable field instrument then power consumption may also be an issue therefore the first parameter we investigate is operating temperature and we consider a range from room temperature to body temperature.

Progesterone is a steroid hormone involved in female menstrual cycle, pregnancy and embryogenesis of humans and other species. It is one of a number of substances said to cause hyperactivation of mammalian spermatozoa and its presence may therefore affect the time of flight; the effect of adding progesterone to the swim medium is reported.

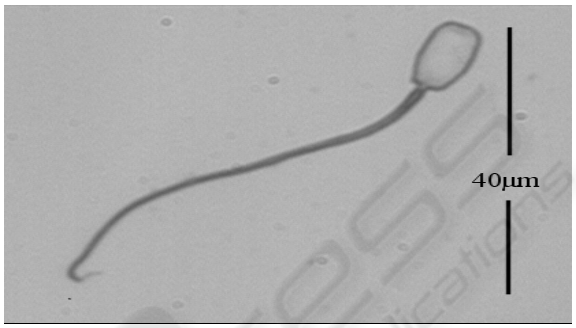

Figure 1: Magnified view of a boar sperm.

2 EXPERIMENTAL

Figure 2 shows a schematic diagram of the experiment. This consists of an inlet port to a channel filled with phosphate buffered saline (PBS) buffer. At the other end of the channel is a quartz crystal followed by a vent to air preventing pressure changes being recorded in the QCM response when a semen sample is added. Sperm are introduced at the inlet port and are self propelled through the channel to the QCM where they are detected. A volume of 20μl of the semen was used and added using a Gilson pipette. The channel length was set to approximately 14.5 cm and contained 4ml of PBS; note that for any practical field instrument the swim channel length could be considerably reduced to give an analysis time under 5 minutes. The sensing element in the experiments was a 5MHz AT-cut quartz crystal (Testbourne 149211-1). A Maxtek PLO-10 phase lock oscillator was used to drive the crystal and the resonant frequency was measured with an Agilent universal frequency counter interfaced to a computer.

To sense the sperm it was necessary to get them to adhere to the surface of the QCM. To achieve sperm adhesion to the crystals they were coated in either Poly-L-Lysine (Sigma-Aldrich) or cysteamine (Sigma-Aldrich). Crystals were initially cleaning with ethanol then ozone treated for 30 minutes. They were then placed in either poly-L-lysine (as supplied), or a cysteamine solution of 1mmol in toluene and left overnight. The devices were then

washed in PBS buffer to remove any excess. The cysteamine coating were found to be the most reliable method of binding the sperm to the surface as poly-L-lysine shows significant variability from batch to batch.

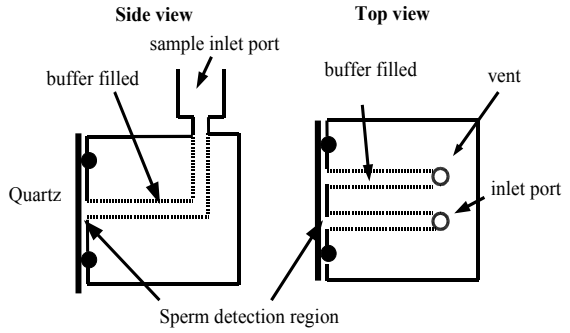

Figure 2: Schematic diagram of swim channel.

To provide temperature control to the experiments the swim channel was housed inside an Octagon 10 incubator. This allowed the temperature to be controlled so as to investigate the effect this would have on the sperm ToF. For these the temperature was varied from 23-45°C. The incubator also allowed the temperature to be kept constant across all the progesterone experiments.

When looking at the effects of progesterone, (Sigma-Aldrich) different concentration were added to the PBS in the swim channel. Firstly 15.7mg of progesterone was dissolved in 50ml of ethanol. This mixture was diluted in PBS at concentration of 20-90µmol.

The porcine semen was supplied by a commercial artificial insemination centre (JSR Genetics, Driffield, UK). The semen was received already mixed with a dilutant (Androhep), cooled to a temperature of 17°C and packaged in plastic bottles. The androhep allowed the semen to be stored for up to 5 days at ambient temperature. However, this does result in the concentration of semen in the mixture being quite low. To get a more concentrated sample a centrifuge was used to separate the sperm from the androhep. To achieve this, sealable microtubes were filled with 50µl of the androhep and semen mixture and these were centrifuged for 40 seconds. This resulted in the sperm being concentrated at the bottom of the tube. The androhep was removed with the pipette and 50µl of PBS was added to one of the tubes. The PBS and sperm were mixed together, this mixture was removed with the pipette and added to the next tube and the process was repeated for all 15 tubes. What was left was a more concentrated sperm sample mixed with 50µl of PBS.

3 RESULTS AND DISCUSSION

Figure 3 shows the QCM frequency response to sperm binding on the surface. The arrow shows the time at which the semen sample was introduce to the inlet of the swim channel. The arrival of the sperm is signified by a decrease in the frequency of the sensor with the fastest ToF of approximately 20 minutes.

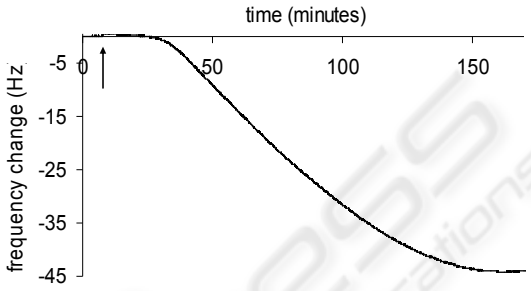

Figure 3: Graph showing the frequency decrease indicating sperm arrival.

The frequency continues to drop as more sperm make their way to the QCM and bind to the surface. This continues for another 120 minutes until further arrival of motile sperm finishes.

Using the previously determined sperm effective mass and taking the rate of frequency change from figure 1, the Sauerbrey equation can be used to derive the rate of sperm arrival and this is shown in figure 3. For use in a screening application, a simple threshold number of detected sperm would be required however this demonstrates that quantitative analysis is also possible with this instrument.

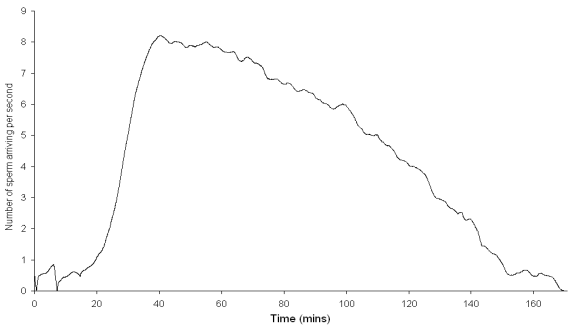

Figure 4: Number of sperm arriving at the QCM over the course of the experiment.

Figure 5 is a plot of the ToF of the sperm against the temperature of the environment. The results show a decrease in the ToF as temperature increases with almost a 50% fall between room temperature and body temperature. The scatter observed can be attributed mainly to the experiments being performed a differing lengths of time from the

receipt of the samples and the quality of the semen degrades over time.

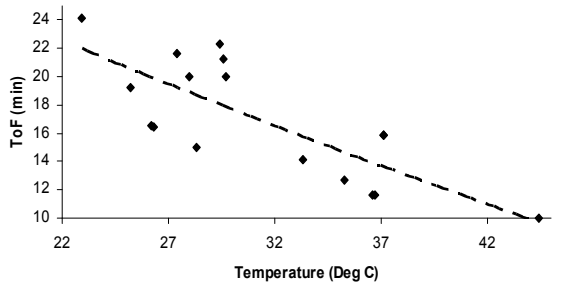

Figure 5: The time of flight for fastest sperm arrival as a function of temperature.

To further speed up the measurement, progesterone was used to cause hyperactivation in an attempt to decrease the sperm ToF; the results of this are shown in figure 6. Comparing the non-progesterone experiments with the progesterone ones we see a significant decrease in the ToF of the sperm however for the full range investigated there was little effect from the progesterone concentration.

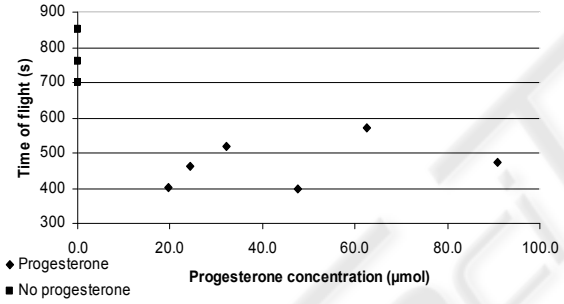

Figure 6: Time of flight as a function of progesterone concentration.

4 CONCLUSIONS

We have demonstrated that a time of flight technique with an acoustic wave sensor provides a viable method for determining the quality of a semen sample both as a screening technique and as an analytical tool. The cysteamine coating on the QCM proved to be the more reliable method of binding the sperm to the surface. Experiments varying the temperature showed a general decrease in ToF as temperature is increased suggesting that body temperature would be the optimum value. The presence of progesterone also reduces the ToF however this was not concentration dependent over the range investigated. Whilst the laboratory based instrument reported here used commercial sensor crystals, the cost of quartz crystals employed more generally in electronic oscillator circuits are inexpensive and are still offer a mass sensitive surface. Using such crystals, pre-treated with cysteamine, would reduce costs sufficiently to offer the possibility of a disposable element. With a modification to the swim channel length to bring down the measurement time, this technique then becomes a powerful tool for routine monitoring of animal reproductive health and investigating factors that affect the semen motility of animal.

REFERENCES

Mortimer, S. T., 2000, CASA - Practical Aspects, J.ournal Andrology, 21, p.515-524

Christensen, P., Boelling, D., Pedersen, K. M., Korsgaard, I. R., & Jensen, J., 2005, Journal of Andrology, 26

Sauerbrey, G., 1959, Zeitschrift für Physik, p.155-206

Da Silva, L. B., Trebes, J. E., Balhorn, R., Mrowaka, S., Anderson, E., Attwood, D. T., Barbee, T. W., Brase, J., Corzett, M., Gray, J., Koch, J. A., Lee, C., Kern, D., London, R. A., MacGowan, B. J., Matthews, D. L., & Stone, G., 1992, X-ray laser microscopy of rat sperm nuclei, Science, 258, p.269-271

Kanazawa, K., & Gordon, J. G., 1985, Frequency of a Quartz Crystal Microbalance in contact with a liquid, Journal of Analytical Chemistry, 57, p.1770-1771

Newton, M. I., Evans, C. R., Simons, J. J., & Hughes, D. C., 2007, Semen quality detection using time of flight and acoustic wave sensors, Applied Physics Letters 90(15)