Assessing sperm motility using acoustic plate mode devices

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***Abstract*— In this work we report a simple time of flight technique for assessing the number and motility of pig sperm in a semen sample. Acoustic plate mode (APM) devices have been employed as the sensor element at the end of a channel down which sperm swim. Combining the APM data with conventional microscopy, an estimate of 18.9±4.8pg for the pig sperm effective mass at 52MHz APM devices is derived.**

I. Introduction

Accurate and precise determination of viable sperm concentration is an important issue for the Artificial Insemination (AI) industry. AI of farm animals is common practice in the agriculture industry with more than 100 million inseminations performed globally each year. Crucial to the success of insemination is not only the number of sperm in a semen sample but also the motility of the sperm. Motility is the term used to describe the motion and activity of sperm in a semen sample. Counting chambers or haemocytometers are used for routine sperm counts. However, multiple measurements are required to achieve an acceptable precision and accuracy which makes the procedure time consuming. The main disadvantages of these methods are the inaccuracy due to the rapid movement of the cells at high magnification and the tedious nature of the work for the human operator. A more objective assessment of sperm motility can be achieved with a laboratory based instrument called a computer-assisted semen analyzer CASA [1] which can give a measure of different aspects of sperm movement. The combination of flow cytometry and fluorescent staining [2] provides a technique to analyze thousands of sperm per sample and achieve a higher precision than is obtained with a CASA systems. The drawback of these technologies is the price of equipment and the need for a skilled operator. In this work we report a simple time of flight technique using acoustic plate mode (APM) devices that has the potential to be an inexpensive field instrument for assessing the number and motility of sperm immediately prior to insemination.

II. Experimental

The APM devices were fabricated on 36^o rotated Y-cut X propagating lithium tantalate of thickness between 530-540 µm. The interdigital transducers (IDT) were of a double-double design with 10µm finger widths and spaces, 50 repeat patterns, a 2000µm aperture, a path length of 8mm giving a fundamental frequency of 52MHz. The fingers consisted of 40 nm of titanium followed by 200 nm of gold deposited by sputter coating. Both faces of the APM device were coated in poly-L-lysine by initially cleaning with ethanol, then ozone treated for 30 minutes. The devices were then placed in poly-L-lysine solution overnight; the devices were then washed in the PBS buffer to remove any excess. The face of the device not containing the IDT's was used as the sperm detection region. The APM device was used as the feedback element in an oscillator circuit consisting of cascaded amplifiers

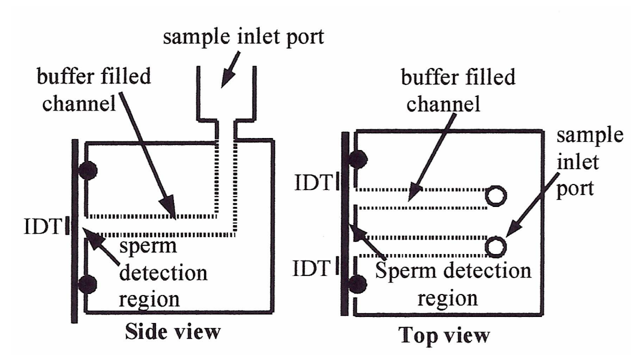

Figure 1. Schematic diagram of experimental arrangement

(Minicircuits ZFL-500LN), a 50MHz high pass filter and 150MHz low pass filter (Minicircuits BHP-50 and BPL-150), a power splitter (M/A com T1000), a pi phase shifter and a frequency counter (Agilent 53132A) interfaced to a microcomputer. Figure 1 shows a schematic diagram of the test cell, sperm were introduced at the inlet port and move through the channel to a sperm detection region comprising the APM device with sperm adhesive poly-L-lysine coating. The channel length was set to approximately 6.5 cm which would mean that the earliest expected arrival of the first sperm would be after 14 minutes [3,4] and this would be followed by a distribution of arrival times. As the sperm have to be motile to travel to the APM device, the time of arrival is a measure of their motility. Pig semen samples were supplied by a commercial artificial insemination centre (JSR Genetics, Driffield, UK). Prior to dispatch the semen was mixed with a dilutent (Androhep), cooled to 17^{o}C packaged in plastic bottles, and delivered by overnight postal service. This medium is suitable for up to 5 days storage at ambient temperature.

III. RESULTS AND DISCUSSION

Figure 2 shows a typical change in frequency for a period of 110 minutes; a 0.2ml pig semen sample was introduced at 31 minutes. At 19 minutes from the introduction of the semen a fall in frequency is observed which is completed after a further 23 minutes and shows a frequency decrease of 1780±160Hz. Josse et.al. have reported that the sensitivity for a 52 MHz APM device is around 73.3Hz/ng mm^{-2} [5].

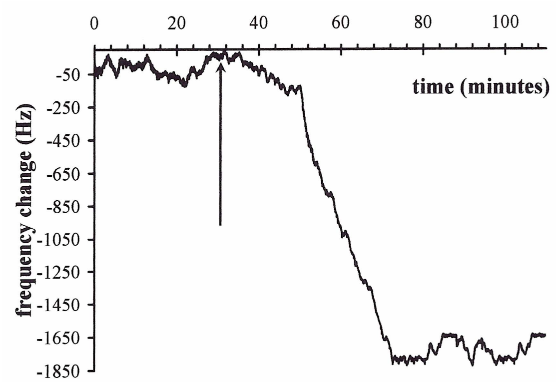

Figure 2. Frequency change of APM device following the semen sample introduced at 31 minutes as shown by the arrow.

Using this sensitivity value and the frequency fall observed, the mass of sperm attached to the sensing area of the APM is 390±35ng. Estimation of the number of attached sperm was made using a poly-L-lysine coated glass slides in place of the APM one hour after introducing the sperm. The slides were checked using conventional microscopy to identify the sperm concentration present. An area of 0.36mm x 0.49 mm on the glass slide at the end of the flow cell gave 237±40 sperm and this equates to 21500±3600 sperm in the path between the IDT's of the APM device.

Figure 3 is a microscope image of a porcine sperm showing that the head is approximately 8μm and overall length of 50 to 60 μm. Given that the penetration depth of acoustic waves in water at 52MHz is only 78nm and will only probe a small part of the attached sperm, we have considered if the attachment of sperm may be approximated by the Sauerbrey equation using an effective mass. In a previous report we have used 5MHz quartz crystal microbalance [6], and fitted the resonance curve with a Butterworth van Dyke model to extract the crystal resistance with and without attached sperm. This showed that little change occurred in the crystal resistance due to the sperm attachment even though a frequency shift was observed. This confirmed that a simplified model based on the Sauerbrey equation and a sperm effective mass could in practice be appropriate. Previous studies have estimated a dry head mass of 13pg [7] and up to 70% of the sperm mass to be made up of water [8]. Using the estimate for the number of sperm and the frequency change of the APM, the effective mass of pig sperm on a 52MHz APM device is 18.9±4.8pg.

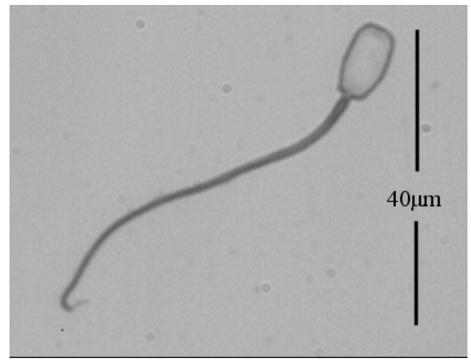

Figure 3. Microscope image of porcine sperm

IV. CONCLUSION

In this article we have demonstrated that acoustic plate mode devices may be employed as the sensor element for a time of flight method to determine sperm motility in a semen sample. One of the main issues that require addressing before the device would be suitable for routine screening is the shelf life associated with the poly-L-lysine coating, preliminary test suggest that cysteamine appears more promising but require further investigation.

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