Semen quality detection using time of flight and acoustic wave sensors

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Abstract

We report a real-time technique for assessing the number of motile sperm in a semen sample. The time of flight technique uses a flow channel with detection at the end of the channel using quartz crystal microbalances. Data presented suggests that a simple rigid mass model may be used in interpreting the change in resonant frequency using an effective mass for the sperm.

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Artificial insemination of farm animals is common practice in the modern agriculture industry with more than 100 million inseminations performed globally each year. Crucial to success is both the number and motility of the sperm in the semen sample. The most common method for evaluation of sperm concentration and motility involves a direct observation through a microscope using counting chambers or haemocytometers. 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 computer-assisted semen analyzer (CASA)[1], however this is a laboratory based instrument which, from program settings, can give a measure of different aspects of sperm movement. The combination of a fluorescent staining and flow cytometry[2] provides a technique to analyze thousands of sperm per sample and achieve a higher precision than is obtained with microscopic assessment or 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 wave devices that has the potential to be an inexpensive field instrument for measuring number and motility of sperm.

Acoustic wave sensors are based on the ability of acoustic wave devices to detect very small changes in mass attached to their surface and usually contain a sensitizing layer that can recognize and bind the species we want to detect onto the mass sensitive surface. The most widely used acoustic wave device for sensor applications is quartz crystal microbalance

(QCM). The Sauerbrey equation[3] relates the change of the crystals resonant frequency is proportional to the change in rigid mass on the crystal surface; for AT cut quartz this gives $\Delta f = -2.26 \times 10^{-6} f^2 \Delta m/A$ 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); the constant comes from the crystal materials properties. A well-designed oscillator circuit can still resonate a crystal immersed 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[4].

Porcine semen samples were supplied by a commercial artificial insemination centre (JSR Genetics, Driffield, UK). Prior to despatch 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. A flow cell was fabricated to use two polished 5MHz quartz crystals with gold electrodes (Testbourne 149211-1) as the sensing and reference as shown in figure 1a. The sensing crystal was coated with poly-L-lysine and the reference left uncoated. To prepare the poly-L-lysine coated crystals, they were initially cleaned with ethanol, then ozone treated for 30 minutes. The crystals were then placed in poly-L-lysine solution overnight; the devices were then washed in the PBS buffer to remove any excess. The blank crystals were cleaned with ethanol followed by PBS buffer. The crystals were used with Maxtek PLO10

phase lock oscillators interfaced to a computer and data was collected 36 times a minute. A channel length was approximately 6cm to give a typical time of flight of around 10 to 15 minutes from the literature values for sperm velocity[5,6] and the swim up medium used was PBS buffer.

Figure 1b shows the change in frequency for the reference crystal (upper line) and the sensing crystal (lower line) for a period of 55 minutes; a 0.2ml semen sample was introduced at 16 minutes. The reference crystal shows a small positive drift in frequency of 5Hz over the measurement period with a small deviation as the semen was introduced. The poly-L-lysine coated crystal shows a negative drift of around 0.3 Hz per minute prior to and after the sperm detection. At 12 minutes from the introduction of the semen a fall in frequency is observed which is completed after a further 8 minutes and shows a frequency decrease of approximately 15 Hz; the average frequency shift for repeated measurements on the same batch of semen gave 35.2 ± 24.7 Hz. Confirmation that the signals observed are from the attachment of sperm were made by using the flow cell with uncoated and poly-L-lysine coated glass slides in place of the quartz crystals for different periods of time after introducing the sperm. The slides were checked using conventional microscopy to identify the sperm concentration present. No attachment was observed on the uncoated slide. Measuring 18 separate areas of 200 x 250 μm on the poly-L-lysine coated slide at the end of the flow cell gave 166 ± 88 sperm after one hour; this corresponds to 440k±233k sperm attached to the sensing electrode area of 1.327 cm^2 of the QCM.

To assess if significant dissipation was taking place following the sperm attachment, a network analyser (Agilent 7431ET) was used to record

the reflected power resonance peak for a poly-L-lysine coated crystal in PBS. The crystal holder was arranged to have the crystal horizontal so that gravitational settlement could occur and hence a maximum attachment of sperm could be achieved. Figure 2 shows the spectrum for the crystal in PBS buffer (line 1), for the crystal after a 0.2ml charge of semen had been added and left for an hour (line 2) and after the semen had been washed out and clean buffer included (line 3). The frequency of the peak shown on the network analyser peak is expected to track the frequency that would be achieved using the oscillator circuit however, if significant dissipation occurs i.e. a non-Sauerbrey relationship hold, then it would be expected that in addition, the peak would broaden. The difference between line 1 and line 2 shows such a broadening is obtained with the gravitational settlement of the sperm onto the crystal surface. However, after surplus sperm are removed the resonance of line 3 is as sharp as line 1. In a Butterworth - Van Dyke (BVD) equivalent circuit model for the crystal, the crystal resistance R represents energy loss processes, and a change in this shows departure from a rigid mass attachment model. The data shown in figure 2 was fitted using a BVD model. For the initial PBS buffer R=368.4Ω, one hour after introducing the sperm R=384.2Ω and after the excess have been removed and clean buffer introduced R=367.5Ω. This suggests that a simplified model based on the Sauerbrey equation and a sperm effective mass may 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]. Applying the Sauerbrey relationship to the average frequency change observed and using the estimate for the number of sperm attached this corresponds to a pig sperm effective mass of 4.2 ± 3.7pg.

In summary, we have demonstrated a real-time technique for assessing the number of motile sperm in a semen sample and that a simple rigid mass model may be used in interpreting the change in resonant frequency using an effective mass for the sperm. The use of higher frequency acoustic wave devices such as acoustic plate modes would offer increased sensitivity.

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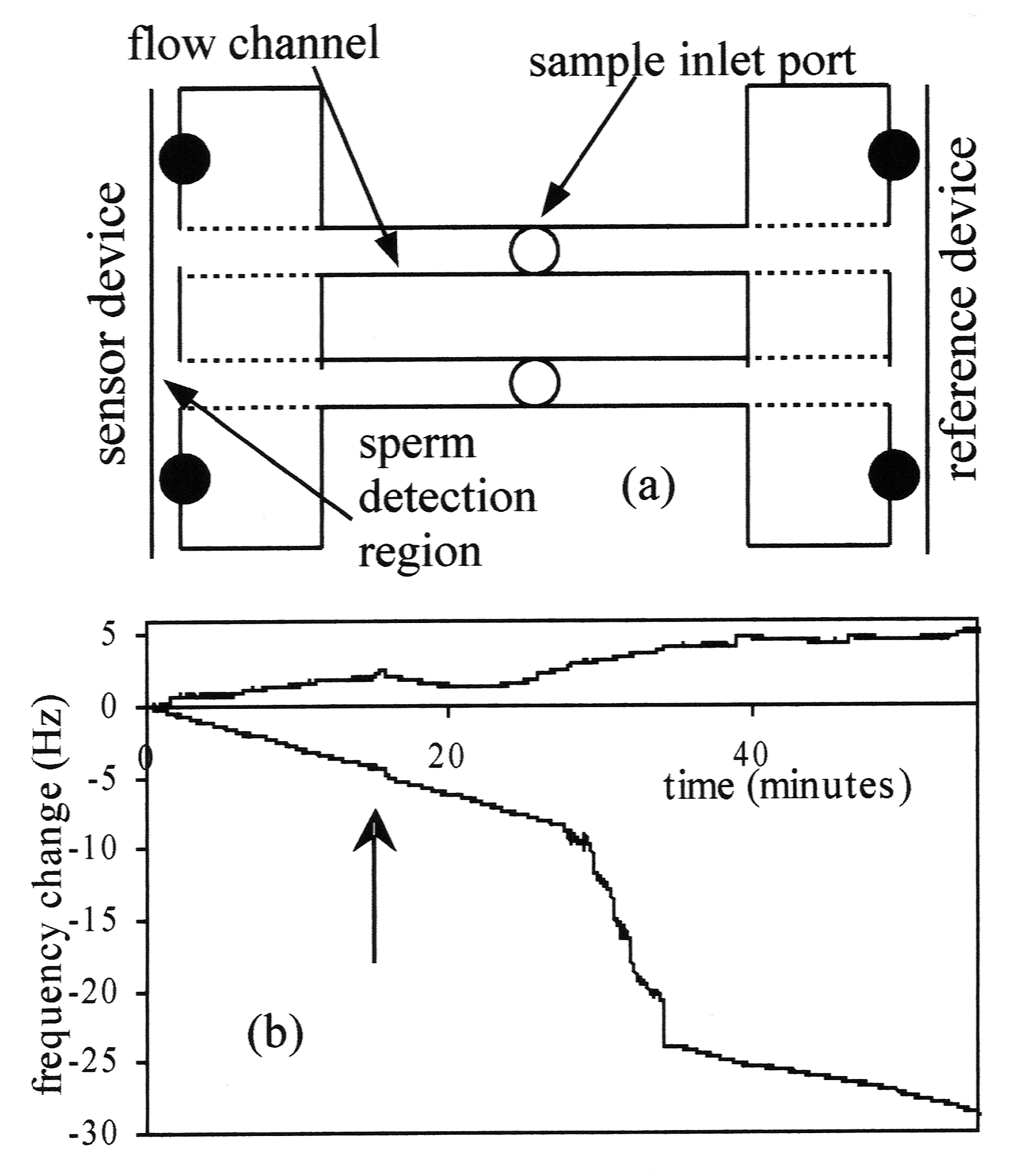
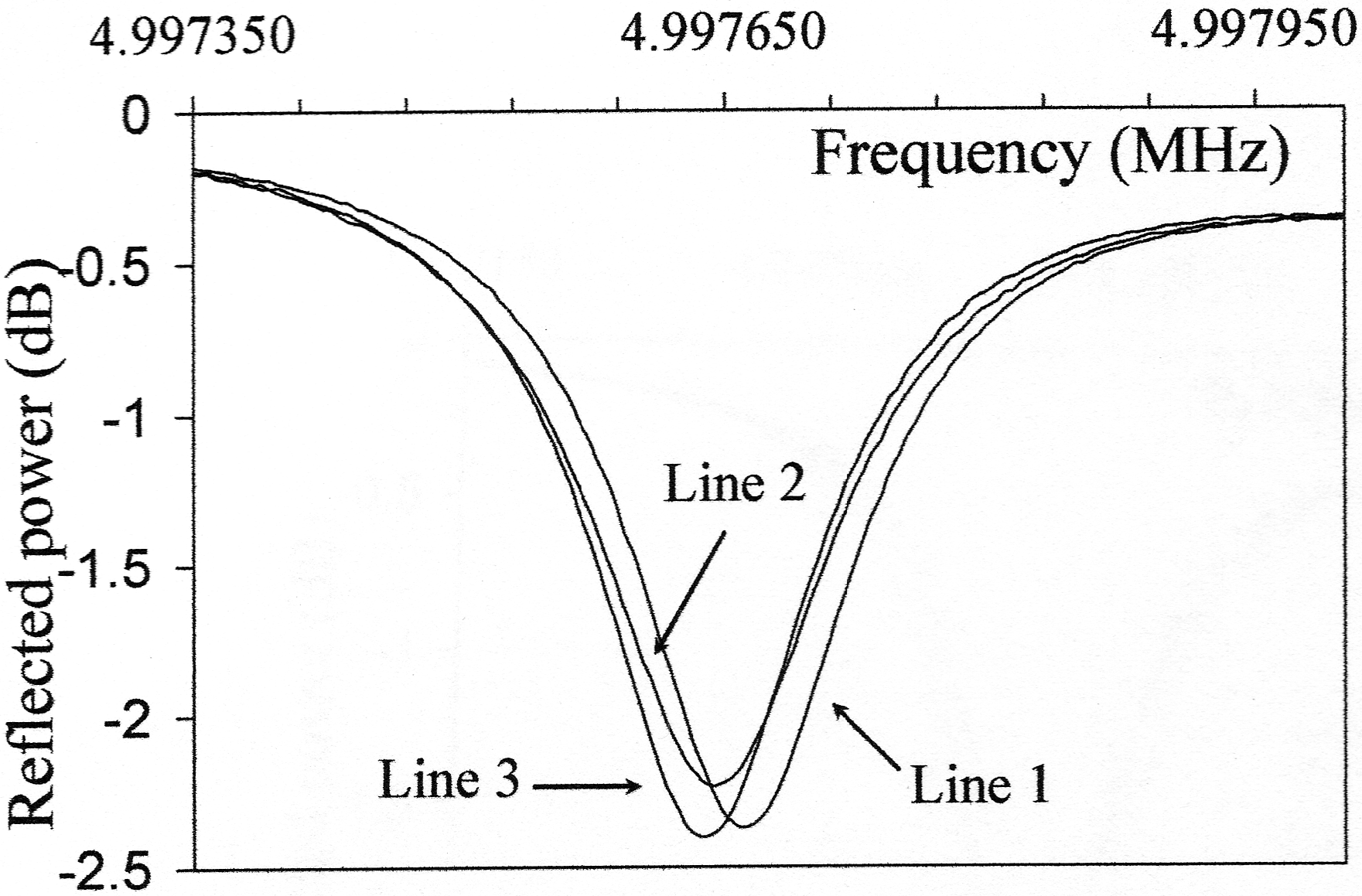
Figure captions

Figure 1.(a) schematic diagram of experiment (b) Frequency change as a function of time for the reference (upper line) and sensing (lower line) crystals. The arrow shows the time at which the sperm sample was introduced to the inlet port.

Figure 2. Spectrum of crystal with PBS buffer only (line 1), with sperm after 1 hour (line 2) and with excess sperm removed and fresh buffer (line 3).


flow channel
sample inlet port
sensor device
reference device
sperm
detection
region
(a)
5
0
-5
-10
-15
-20
-25
-30
0
20
40
time (minutes)
frequency change (Hz)
(b)


4.997350
4.997650
4.997950
Frequency (MHz)
0
-0.5
-1
-1.5
-2
-2.5
Reflected power (dB)
Line 2
Line 3
Line 1