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PROVISIONAL SPECIFICATION

Title: A Method And Apparatus For Selecting Cells

I, ***Frontin-Rollet, Andrew***,
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do hereby declare this invention to be described in the following statement :

A METHOD AND APPARATUS FOR SELECTING CELLS

FIELD OF INVENTION

The present invention generally relates to a method and apparatus for selecting desired cells, or parts of cells, preferably, desired sperm cells and more particularly relates to a method and apparatus for orientating, selecting and retaining desired cells or parts of cells.

BACKGROUND OF THE INVENTON

There has been a long felt need for a reliable, qualitative and cost-effective method for selecting sperm which may be used to produce animals of a desired sex.

In particular, in the livestock industry farmers or breeders require cows, pigs, sheep, goats, deer, buffalo, horses, etc which are of a preferred sex. For example, bulls are of limited use to a dairy farmer, whereas pig farmers have long been aware that the female pig grows at a faster rate than their male counterparts.

Similarly, cattle and sheep farmers understand only too well that the males of these species produce meat at a faster rate than females.

In mammals the egg carries only the X chromosome whereas the sperm carries either an X or a Y chromosome. The sex of progeny is therefore determined by the sperm cell. When a sperm and an egg are combined and the sperm carries the X chromosome the offspring is female (XX). However, if the sperm carries the Y chromosome, once combined with the X chromosome carried by the female the resultant offspring will be male (XY).

In sperm there is a known difference in DNA content between the X (larger) and the Y (smaller) sperm of for example 3.4% in pigs, 3.8% in cattle and 4.2% in sheep. This measurable difference can be used to determine the sex of the sperm, that is, if it is an X chromosome (female) or if it is a Y chromosome (male) bearing sperm.

The prior art discusses and provides for methods for sorting mammalian sperm into X and Y populations. However the only reliable methods are based upon the measurement of the DNA content of individual sperm. These methods invariably use fluorescence measurement to detect what are essentially small differences between the X and Y sperm, wherein the sperm pass single file through a system which measures the DNA content of individual sperm.

Some techniques have been expanded to use a bevelled sample injection tip and a second fluorescence detector in a forward position. The second fluorescence detector is adapted to determine the orientation of flat oval shaped sperm with respect to the first detector as they pass through the system..

In both cases the magnitude of fluorescence is being measured.

Further adaptations allow for those unwanted sperm to be gated and pass through as waste and discarded.

The prior art therefore describes a flow cytometric system, which requires two separate measurements of the magnitude of fluorescence of the sperm cell, one to determine the sex of the sperm, the other to determine the sperm's orientation. Those skilled in the art would recognise that due to the morphology of sperm cells (flat ovoid shape) and extremely high refractive indices, it is not possible to accurately measure the DNA content of sperm unless said sperm are correctly oriented to the DNA fluorescence detector.

This method of analysis is expensive – and do not always provide for routine efficiencies much in excess of 80%, although 95% efficiencies have been reported.

Surprisingly, the present inventor has found that a process wherein the orientation of a sperm cell is determined by passing light using optical phase contrast techniques through a sperm cell of interest provides for improved efficiencies and increased reliability in the results obtained. In other words orientation of sperm cells – the correct orientation defining whether a result should be accepted for further analysis – can be determined by measuring non-fluorescent light emitted by a sperm cell.

The measurement of non-fluorescent light has never before been considered as a means to determine the orientation of cells.

Surprisingly, the inventor has further found that there is no need for the cells of interest to be encapsulated or confined within an electrically charged medium during the analysis and collection phase of the process. Previously, once the DNA content of a sperm cell had been determined, the cells were encapsulated in a droplet to which is appended an electric charge, the charge being dependent on the cells DNA X or Y sex chromosome content. The droplets were then separated based upon the charge they received. The present invention simply selects those cells having a desired DNA sex chromosome based upon predetermined parameters programmed in the analyser. If the criteria are met the cell is collected and retained. If the criteria are not met the cell is eliminated by a process of ablative photodecomposition, generally by exposure to a laser.

This invention also teaches the use of a rectangular testing zone located downstream of an orienting nozzle. A cell emerging from the orienting nozzle can be maintained at the correct radial orientation to allow for accurate analysis. The substantially rectangular configuration of the testing zone of the invention has been found to provide for superior accuracy and more reliability in the results obtained. Previous testing processes maintained the cell being measured (although generally correctly aligned) in a circular cross sectional fluid stream or liquid droplet which is of an essentially elliptical or circular configuration as the cell emerged from the nozzle. This configuration, although allowing for increased cell flow rates of a desired orientation, also allows for inaccuracies due to light being refracted from the curved surfaces of the fluid stream or droplets being measured.
Importantly, this rectangular testing zone provides for four flat surfaces. The improvement results in a significant reduction in unwanted refracted light which when curved surfaces are used thus eliminated false readings. As such the provision of four flat surfaces provides for a much-improved reliability over previously disclosed systems.

The present invention comprises therefore at least three components, the aspects of which will be outlined later in greater depth. Firstly, the invention uses phase contrast optics to determine a cells

orientation. Secondly, the invention makes no requirement for the cells of interest to be encapsulated in droplets or otherwise to enable desired cells to be sorted from those that are not wanted. Thirdly, the use of a substantially rectangular testing zone reduces the effects measurement of unwanted light has on the process.

The above features therefore provide significant and surprising advantages over existing cell selection/sorting processes and in particular those processes directed to the sexing of sperm cells.

OBJECT OF THE INVENTION

It is an object of the present invention to provide an improved method and/or apparatus for selecting desired cells, or parts of cells, or is one which will obviate or minimise the foregoing disadvantages or will at least provide the public with a useful choice.

STATEMENT OF THE INVENTION

Accordingly, a first aspect of the invention provides for a method of determining the orientation of a cell in a process wherein said orientation is used to allow for the determination of cell differences due to size, mass, volume or density and whereby the orientation of the cell is determined by measuring non-fluorescent light.

Preferably, the orientation is determined by measuring light using a band pass filter to exclude all light other than from the phase contrast light source/condenser.

Preferably, the method for determining the orientation of the cell does not require the cell to be encapsulated within a droplet.

Preferably, the method for determining the orientation of the cell is used in tandem with a method for measuring the DNA content of the cell.

Preferably, the method for determining the orientation of the cell is used simultaneously with a method for measuring the DNA content of the cell.

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