High throughput phenotypic screening of the human spermatozoon

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The definitive version is now freely available at DOI: https://doi.org/10.1530/REP-21-0372; [2021].

Reproduction

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Journal:	*Reproduction*
Manuscript ID	REP-21-0372.R1
mstype:	Invited review
Date Submitted by the Author:	n/a
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Keywords:	Sperm function, Contraception, Male infertility, drug discovery, high throughput phenotypic screening

Revised paper HIGHLIGHTED Johnston et al., Review, Reproduction November 2021

High Throughput Phenotypic Screening of The Human Spermatozoon

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Running Title:

Key words: Drug discovery/Sperm function/high throughput phenotypic screening/contraception/ Male Fertility

Abstract

Despite recent advances in male reproductive health research, there remain many elements of male (in)fertility where our understanding is incomplete. Consequently, diagnostic tools and treatments for men with sperm dysfunction, other than medically assisted reproduction, are limited. On the other hand, the gaps in our knowledge of the mechanisms which underpin sperm function have hampered the development of male non-hormonal contraceptives.

The study of mature spermatozoa is inherently difficult. They are a unique and highly specialised cell type which does not actively transcribe or translate proteins and cannot be cultured for long periods of time or matured in vitro.

One, large scale, approach to both increasing understanding of sperm function, and the discovery and development of compounds that can modulate sperm function, is to directly observe responses to compounds with phenotypic screening techniques. These target agnostic approaches can be developed into high-throughput screening platforms with the potential to drastically increase advances in the field.

Here we discuss the rationale and development of high-throughput phenotypic screening platforms for mature human spermatozoa, and the multiple potential applications these present, as well as the current limitations and leaps in our understanding and capabilities needed to overcome them. Further development and use of these technologies could lead to the identification of compounds which positively or negatively affect sperm cell motility or function, or novel platforms for toxicology or environmental chemical testing among other applications. Ultimately, each of these potential applications is also likely to increase understanding within the field of sperm biology.

Introduction.

Recently, there have been several significant developments in male reproductive health that have not only increased our understanding of basic mechanisms but also opened exciting areas of research. One is uncovering how epigenetic modification of the paternal gamete can potentially have profound effects on the health of subsequent generations (Bodden, et al. 2020, Lismer, et al. 2021). Another is understanding how male reproductive health is increasingly viewed as a sentinel marker for somatic health issues (Choy and Eisenberg 2018, Del Giudice, et al. 2021) and, the health of subsequent offspring (Kasman, et al. 2020). However, there are two key areas where progress remains limited namely (1) the diagnosis of and non-Medically Assisted Reproduction (MAR) treatment of men with sperm dysfunction (2) development of new male contraceptives.

This review outlines these two areas including discussing the driving requirement for progress. A common roadblock limiting development, namely the relatively rudimentary understanding of the underlying physiology and function of the mature spermatozoon is presented. The focus is discussing the potential uses of a High Throughput Phenotypic Screening assay of the mature human spermatozoon. This can be used as a platform technology to devise new diagnostic tools and non-MAR therapies, as a tool for the development of new targets and drugs for male contraception and to improve our underlying knowledge of the cell.

Background

Sperm dysfunction, diagnosis, and non-MAR Treatment

A plethora of studies demonstrate that our understanding of the physiology of the mature human spermatozoon is quite limited and this is a significant roadblock to the development of badly needed diagnostic and therapeutic tools (Barratt, et al. 2017, Barratt, et al. 2021, De Jonge and Barratt 2019, Schlegel, et al. 2021a, b). Consider for example the development and use of sperm function assays. There is clear information that classical sperm function tests such as the generation of reactive oxygen species (ROS) and assessment of subsequent damage to the cell have significant clinical value [see reviews (Aitken & Bakos, 2021, Oehninger and Ombelet 2019)]. However, these assays have yet to be translated into robust tests that are routinely used in the clinical setting. Why? There are several reasons, but primary amongst these is our relatively low knowledgebase of the mature spermatozoon and what separates function from dysfunction at a phenotypic level within these assays. One example illustrates this - the complexities of ROS generation and the assessment/impact of ROS damage. Thirty years ago, seminal work from John Aitken and colleagues (Aitken, et al. 1991) provided clear evidence that generation of ROS in a semen sample had a negative impact on the chances of achieving an in

vivo pregnancy in 139 sub-fertile couples. A plethora of subsequent experiments unveiled the complexity of ROS generation as a 'friend and foe' to the spermatozoon and, the challenges of assessing subsequent damage to the cell. Consequently, developing an assay(s) that could easily reflect pathological ROS generation and damage with clear, cut-off values and reference ranges, is not simple. Suffice it to say we still do not have sufficient detailed biological knowledge to illuminate clear diagnostic assays. John Aitken succinctly summarised the reality of the situation as there being 'rampant uncertainty' about which assays to use (Aitken 2021).

A detailed understanding of the physiology of a sperm cell is not only important for the development of diagnostic tools but also to develop rational non-MAR treatment. For example, to successfully use antioxidants for the treatment of sperm dysfunction its first necessary to demonstrate that oxidative stress is the cause of the infertility. The continual unsuccessful use of antioxidants for male infertility is testament to the fact that we have yet to solve this conundrum (Aitken 2021).

Male contraception.

Remarkably, there remain only two well established methods of male contraception namely vasectomy and condoms both of which have inherent disadvantages limiting global utilization. Progress in male hormonal contraception has been slow particularly considering that promising trials were published 25 years ago (World Health Organization Task Force on Methods for the Regulation of Male Fertility 1996) and currently, despite much discussion, there are no products on the horizon (Reynolds-Wright and Anderson 2019, Thirumalai and Amory 2021). Moreover, whilst there has been significant activity to elucidate potential key targets for non-hormonal contraception, high quality human targets have yet to be realized (Long, et al. 2021). Although there are possibilities for targets allowing interruption of spermatogenesis and/or manipulation of the epididymal environment, of particular concern is the scarcity of new targets in the mature human spermatozoon.

As indicated, a fundamental challenge in andrology remains the paucity of knowledge of the physiological workings of the normal (and dysfunctional) spermatozoon. Although progress has been made for example using proteomics or electrophysiology, our discipline still lacks real intensity in these areas. For example, we don't have a detailed understanding of the quantitative protein composition of the spermatozoa, including post translational modifications, and protein interactions [cf sea urchins (Trötschel, et al. 2020)]. Electrophysiological data is still limited on patient samples (Brown et al., 2019) and single cell analysis and imaging is only recently gaining traction (Kandel, et al. 2020, Moscatelli, et al. 2017, Nandagiri, et al. 2021). The power of technology is impressive but uptake and overall development in the discipline of human sperm biology has been relatively slow.

High throughput phenotypic screening

High throughput phenotypic screening in general biology

Phenotypic screening is a target agnostic approach where phenotypic responses to compounds are directly observed. Therefore, compounds that produce a desired phenotype can be identified even when knowledge of molecular targets are limited. Further, screening can be carried out to probe different phenotypes, that may have different physiological relevance. However, care must be taken that the phenotypic assay is relevant to the disease context in the patient (De Rycker, et al. 2018). To achieve this, usually it is necessary to develop screening cascades with a variety of secondary assays (Field, et al. 2017).

Compounds that produce phenotypic responses, or "hit" compounds can be used to identify novel targets, and/or can be modified to modulate the phenotype further. Phenotypic screening is a common approach in drug discovery (Gilbert 2013, Moffat, et al. 2017) and has been successfully used to discover several new compounds for major disorders and diseases e.g. fragile X syndrome (Kaufmann, et al. 2015), hepatitis C (Gao, et al. 2010), cryptosporidiosis (Jumani, et al. 2019, Love, et al. 2017, Love and McNamara 2021), and malaria (Antonova-Koch, et al. 2018, Baragaña, et al. 2015, Baragaña, et al. 2016).

In addition to identifying potential drug candidates, phenotypic screening can provide insights to improving the understanding of biology of the cells or complex model systems in normal or diseased states, by identifying novel, chemical tools (Moffat, et al. 2017). Phenotypic compound profiles can be created through the generation and analysis of complex and rich datasets that describe a multitude of cellular parameters (e.g. morphology, gene/protein expression levels), or functions (e.g. velocity). These profiles can provide a rationale for the identification of molecular targets (target deconvolution) to determine the mechanism of action of hit compounds (Chandrasekaran, et al. 2021, Hughes, et al. 2021, Ziegler, et al. 2021). Whilst target deconvolution of hits can be challenging, this provides valuable information in understanding the fundamental biology of the cell, for medicinal chemistry optimisation of drug hits and leads, and the potential clinical use of a compound.

As a platform for drug discovery and for furthering molecular understanding, the inherent advantages of the phenotypic screening approach are readily translatable to the human spermatozoon. Several functional and clinically relevant in vitro assays, such as sperm motility, acrosome reaction, and intracellular calcium flux assays, have the potential to form a basis for phenotypic screening assays.

Development of Phenotypic screening for human spermatozoa

Development of a high-throughput phenotypic assay for the assessment of human sperm function brings significant technical obstacles. For example, the system must be largely automatic (require minimal labour input), requiring sophisticated robotics, and directly assess the effects of numerous (thousands) of compounds on sperm cell function within a short time. It must also be based on robust (good Z' score) and reproducible assays, with a good signal to noise ratio and a measurable end-point. We have addressed these challenges and developed a high-throughput phenotypic platform (Figure 1), to measure motility (Gruber, et al. 2020), as well as other key attributes of the human spermatozoon (Figure 2). The platform utilises a fully automated robotics platform to carry out primary screening assays in a plate-based format and resulting data analysis can be largely automated to process the large amounts of data these high-throughput screens produce. The flexibility and automation allows for multiple aspects of sperm function to be interrogated with different assays (Figure 2). This platform has been utilized to screen several libraries to demonstrate that the assay is scalable, provides consistent biological data compared to traditional sperm function assays such as CASA, and rapidly samples sufficient numbers of cells. In addition, the platform also provides the opportunity for further developments; to adapt the screening platform to other assays and purposes. For example, the addition of conditions that support capacitation, the flexibility of compound addition and incubation times (Figure 1) or the opportunity to adapt the technology to answer different questions with different physiologically relevant environments.

Now that a high-throughput phenotypic assay is available what opportunities arise? We identify five areas to illustrate the potential power of this technology. Although these are presented as separate entities, they are not exhaustive or mutually exclusive and there is significant overlap such that data from one area inform another.

1. *Identification of compounds negatively affecting human sperm function: male contraception.*

A premise for a phenotypic screen is to have an assay that has physiological/disease relevance. Sperm motility is ideally suited as it is easy to measure and is related to both in vivo and in vitro fertility (Tomlinson, et al. 1999). Consequently, a phenotypic assay based on sperm motility is a logical approach for the assessment for contraceptive purposes. This is our primary strategy for the identification of compounds that adversely affect sperm motility, as part of the Bill and Melinda Gates Foundation Contraceptive Program. https://gcgh.grandchallenges.org/challenge/accelerating-discovery-non-hormonal-contraceptives

Several different strategies can be adopted to achieve this goal. For example, the assessment of libraries enriched or specifically containing repurposing compounds such as the ReFRAME library (https://reframedb.org/; (Janes, et al. 2018). A primary advantage of this approach is that, if a compound(s) of interest is identified then it is likely to be accompanied by significant safety/clinical development data thus facilitating more rapid development and potential translational impact. Screening of repurposing libraries is a successful approach as exemplified by its use in anti-cryptosporidial (Janes, et al. 2018) and anti-leishmanial drug development (Patterson and Fairlamb 2019) that have led to rapid progression of compounds into clinical trials, clofazimine for cryptosporidiosis and fexinidazole for visceral leishmaniasis.

A complementary strategy could include using chemogenomic libraries. These are known small molecule pharmacological agents against a broad spectrum of annotated targets/pathways. Thus, the emphasis when using these is not on generation of chemical matter as for a medicinal chemistry program but for generating information on potential targets and informing follow up on target identification (Jones and Bunnage 2017). Examples of such libraries include Sigma library of pharmacologically active compounds (LOPAC1280; www.sigmaaldrich.com), Prestwick Chemical library (www.prestwickchemical.com) both of which have been used in a large number of publications.

Another approach could be the use of pharmacologically focused libraries that contain known inhibitors or chemical scaffolds for target classes such as proteins kinases (Brenk, et al. 2008, Woodland, et al. 2015). Kinases play a significant role in human sperm function and testing these libraries could be helpful in addressing their role. As it's a phenotypic screen and the whole intact spermatozoon is being assessed, a key advantage of such annotated libraries is that not only one or several kinases are examined but the whole kinome is examined, leading to an understanding of kinases in their biologically relevant states.

Following initial screening and hit identification, hits must be confirmed, usually through the re-supply of fresh chemical compound, re-testing, and subsequent testing in a variety of biological and pharmacological assays, as guided by a Target Candidate Profile. The aim of this work is to optimise the compounds to increase potency and improve physicochemical, pharmacokinetic and safety profiles. In the case of sperm, an initial important assay is to check the compounds in a mammalian cell line (counter screen), to ensure that there is a measurable selectivity window between sperm motility inhibition and general cytotoxicity. Further assays include *in vitro* physicochemical profiling such as solubility and metabolic stability in liver microsomes. This screening cascade would also include a series of additional assays, both cellular and animal models, to assess the biological activity of key compounds against sperm and their likely effectiveness as a contraceptive.

Although the use of phenotypic screening of human sperm is still in the early stages, it potentially represents a disruptive technology in the arena. Several interesting preliminary compounds have been identified using the above approaches (Gruber, et al. 2020), and are currently generating potential start points for a contraceptive medicinal chemistry programme. Disulfiram is one example of an identified compound that significantly reduces motility (70% reduction at 10μM) within a relatively short time frame (1 hour). Although not deveopable as a male contraceptive, due to likely side-effects, the identification of Disulfiram indicates that compounds with robust contraceptive activity can be identified using this approach, for either progression into a medicinal chemistry programme or for use as a tool compound for target identification or as a control compound for further assay development.

2. *Identification of compounds positively affecting sperm motility: potential for non-MAR therapy.*

Identifying compounds that increase sperm motility (but do not adversely affect other critical aspects of sperm function) has been a key goal in clinical andrology for decades. If effective and safe, such compounds could be used in MAR treatments such as intrauterine insemination [see (Tardif, et al. 2014)] where the objective would be to temporarily improve the motility of the cell so that there were a higher number of functional cells able to reach the site of fertilisation and/or interact with the egg. The compounds could be added to the sperm cells during the normal routine processing of the sample for insemination and if effective would make treatment more cost effective, less complex, and widely available as treatment became available for more patients.

Whilst the screening system for examining compounds that increase motility is effectively the opposite to contraception it's not simply a case of observing a different readout - increase vs decreases in motility. The systems need to be set up differently as the aims are diametrically opposed. For contraception purposes a fast-irreversible block of motility is the goal. For enhancement of fertility, motility would need to be relatively long lasting rather than a temporary boost and, conversely, it's important not to overstimulate the cell to avoid metabolic exhaustion. As such the assessment time periods are considerable. Also, it is necessary to study the effects in different environments e.g. non capacitating, capacitating conditions to examine if the effect is modified or even negated as the cell is processed and moves toward the egg (Tardif, et al. 2014). Different counter screening is also necessary for example to eliminate undesired adverse outcomes on the fertilising ability of the cells such as avoiding premature stimulation of the AR. Using the phenotypic motility screening approach, in preliminary data, several compound classes have been identified which warrant such further investigation (Gruber, et al. 2021; under review and available as a pre-print).

In this case, a similar library selection strategy to identify compounds, which have a negative effect on sperm function, as in the contraceptive approach, was adopted e.g. chemogenomic and focussed libraries. However, it would be interesting to examine the repurposing of target-annotated libraries as identification of compounds in this area may facilitate a more rapid translation to the clinic, where safety data for the compound already exists or the compound is already in therapeutic use for another indication.

3. Improving our understanding of the functional mature spermatozoon

There are several areas where a phenotypic assay could potentially be utilised to improve our fundamental knowledge of the cell. For example, understanding the role of ion channels. A wealth of data suggests that these are critical for human sperm function but even the basic operation of the most widely studied channel - CatSper – remain somewhat a mystery (Brown, et al. 2019). Furthermore, we don't have a full inventory of the functional ion channel repertoire of the human sperm plasma membrane.

One approach to examine ion channel function in the human spermatozoan would be to screen against large commercially available libraries containing putative or known ion channel inhibitors. Initial experiments could assess effects on, for example, basal motility and kinematics. This would address the question of what role ion channels play in the maintenance of sperm motility. Further experiments could extend to assessing the cells incubated under capacitating and non-capacitating conditions providing important insight into the role of capacitation. Moreover, as assessment of hyperactivation is standard in CASA (Mortimer and Mortimer 2013) it is easily translatable to phenotypic screening. The importance of ion channels in maintenance and induction of hyperactivation could then be examined on a high-throughput scale including assessment of the dynamic nature of these events.

An important issue in the advancement of andrology is a lack of consistency between research groups, and indeed clinics, in sperm handling techniques and in culture media composition. This may, in part, explain some of the uncertainty and conflicting literature in the field (Hernández-Silva, et al. 2020). For example, recent work elucidating the mechanistic role of albumin in activating the proton channel hHv1 and capacitation of human sperm (Zhao, et al. 2021) highlights the importance of sufficient levels of albumin in sperm culture media to fully support capacitation. Experiments carried out in media with differing levels of albumin have the potential to yield differing results due to impaired capacitation. A plate-based screening platform may also provide an approach for examining the phenotypic effects of different culture condition and media additives on the mature human sperm cell in parallel. The scale and flexibility of high-throughput platforms could also aid in

addressing other conflicting data within the field, where there is a particular need to screen large numbers of compounds/samples under consistent conditions, for example the action of endogenous steroids and plant triterpenoids on the regulation of human CatSper (Brenker, et al. 2018, Mannowetz, et al. 2017, Rehfeld 2020).

Understanding is not limited to investigation of the normal spermatozoon. Whilst we have not examined it, the system could be used to test well-defined cohorts of patient samples. For example, when determining effects of compounds to potentially increase motility, do all patients with isolated asthenozoospermia have the same kinetic responses? Moreover, these patients' sample could be assessed under the same conditions (e.g. capacitation timing) determining the dynamics of individual responses.

4. Large scale screening of potential toxicants: environmental/occupational chemicals.

There is a growing body of data on the potential direct effect of environmental chemicals. Although currently this work is largely restricted to examination of outputs such as calcium (Birch, et al. 2021, Rehfeld, et al. 2020, Schiffer, et al. 2014), several small scale studies have indicated direct effects of specific environmental chemicals and mixtures on sperm motility (Grizard, et al. 2007, Pant, et al. 2013, Sumner, et al. 2019). High-throughput examination of sperm motility (and other functional attributes) could provide complementary data on a larger scale and may be particularly relevant where assessing the complexities of interactions between compounds, which require quantum leaps in throughput.

5. Future Method Development:

In addition to the assessment of motility we have developed a phenotypic screen for the AR that uses lectins well established in sperm physiology (Mortimer, et al. 1987). Importantly, we have multiplexed the motility and AR phenotypic assays so that, if required, in one well we can report the activity of a compound on two key aspects of sperm function (Gruber, et al. 2020)**.**

The development of increasing numbers of *in vitro* technologies for cell-based assays further augments the potential benefits of phenotypic screening. In essence, having established a fundamental platform, if a robust and functionally appropriate assay is available, it could be miniaturized and adapted for use in the phenotypic screen. In sperm biology this could range from examination of tyrosine phosphorylation (Matamoros-Volante, et al. 2018) to assessment of membrane potential with the latter recently associated with fertilising capacity (Baro Graf, et al. 2019, Brown, et al. 2016, Molina, et al. 2019).

A physiologically and clinically relevant phenotypic assay is a cornerstone of phenotypic screening. Whist *in vitro* assessment of sperm motility is used clinically and has both diagnostic and predictive value [e.g. (Tomlinson, et al. 1999)] it is assessed in non-viscous media. In contrast, sperm spend their functional life in more viscoelastic environments in the female tract, hence adapting and developing in vitro tests to make them more physiologic may be helpful. For example, adaption of the viscous media penetration assay (Kremer test) that is used as a simple, reliable but very low-throughput method to demonstrate the effect of compounds on sperm motility would be a valid phenotype (McBrinn, et al. 2019, Williams, et al. 2015, Yuan, et al. 2020).

Whilst the focus of this review is on the human spermatozoon, the same technology can also be adapted, and fine-tuned for use on animal spermatozoa. For example, we have modified the high-throughput phenotypic motility screening assay so it can be used on conventional and sex sorted cryopreserved bovine spermatozoa (McBrinn unpublished) opening the technology to research in animal reproduction.

Limitations of phenotypic screening

High-throughput phenotypic screening of the human spermatozoon requires expensive technology that is not routinely available within academic settings. Moreover, any phenotypic assay is by necessity a compromise between the need to rapidly obtain information on large numbers of compounds and having an assay that represents the functions of the cell. For example, media conditions can either be non-capacitating or support capacitation and cells must be prepared and washed for dispensing rather than examining compound exposure in the presence of seminal plasma. Therefore secondary, lower-throughput, assays are required to fully examine the effects of hit compounds.

Moreover, whilst compound identification and effect are the remit of phenotypic screening it's only a platform for further study. Identification of the target (target deconvolution) and detailed mechanisms of action (MOA) studies are usually required, and, in some cases, these can be difficult to achieve. For example, McBrinn *et al.* (2019) and colleagues describe the hurdles in examining MOA of a well-known phosphodiesterase inhibitor of sperm function - Trequinsin. In some arenas, despite numerous years of research, the MOA of compounds can be poorly understood even when these are drugs commonly used to treat disease

Fortunately, along with the complex screening technologies and physicochemical assessments available with high-throughput screening technologies and drug discovery, within andrology further functional assays are available to be adapted for both hit confirmation and targe deconvolution. For

example, adapted viscous media penetration assays can be used to examine the effects on prepared cells or raw semen, and sperm cells subsequent ability to penetrate a physiologically relevant viscoelastic medium (McBrinn, et al. 2019, Tardif, et al. 2014).

For further method development, some biological challenges remain that limit adaptations of phenotypic screening of the human spermatozoon. For AR, while we can assess the effects of compounds on their potential to induce AR, for contraceptive purposes, it would be interesting to see if compounds were able to block physiological induction of AR. However, to achieve this we would need to have a single robust and reliable physiological agonist, which is unfortunately not yet available.

As high-throughput systems aim to automate as much of the workflow as possible, the technology has largely been utilised with adherent cell lines. These, for the most part, can be cultured over several days or even weeks, proliferate, and are well characterised. Some of the major challenges of adapting these technologies to andrology come from the nature of human sperm cells. Sperm cells are highly specialised non-dividing cells, unique in shape and size with specialised organelles that do not actively transcribe or translate proteins. In practical terms, supply of cells is limited by donor or patient numbers and availability and cells cannot be cultured for long periods of time. Their preparation from raw semen is also limited to a time-consuming process that at least so far cannot be automated. Therefore, in all the assays described above, the sperm cells themselves are often the limiting factor, and this must be considered while planning their use in phenotypic screening, or with high-throughput technologies.

Conclusions

In conclusion we have discussed the potential advantages of using a newly available phenotypic screening platform for the human spermatozoa. Work is in its infancy, but it has the potential to facilitate the discovery of new areas of sperm biology to improve our understanding of this highly specialized cell type. Platform technologies such as this will help address key log jams in development of compounds to treat and conversely impair fertility (contraception), improve diagnostics assays and understanding. It will be exciting to see what new avenues this type of investigation bring to sperm biology.

Acknowledgements.

Thanks go to Dr Steve Publicover for critical reading of the manuscript.

Conflicts of interest.

CLRB is Editor for RBMO. No other authors declare a conflict of interest.

Authors Roles:

CLRB designed the concept and framework of the paper and wrote the first draft. All authors contributed to subsequent drafts and to the final editing of the manuscript. All authors approved submission of the final manuscript.

Funding

This work was supported, in whole or in part, by the Bill & Melinda Gates Foundation [INV-007117]. Under the grant conditions of the Foundation, a Creative Commons Attribution 4.0 Generic License has already been assigned to the Author Accepted Manuscript version that might arise from this submission.

386 **Figure legends.**

387 **Figure 1**: The Flexibility of the high-throughput phenotypic screening platform and automation

388 The high-throughput sperm screening platform developed by Gruber *et al*. utilises a 384 well plate
389 format and automation through liquid-handling machines and interchangeable measurement
390 devices. Sperm cells from donor or patients can be observed within the system, and although the
391 focus of platform development has been human sperm, the platform can also be used for the
392 examination animal sperm such as bovine. Depending on the assay format, non-capacitating or
393 capacitating conditions can be used and cells can be exposed to library compounds for defined
394 periods of time, as well as controls and stains appropriate for each application. Interchangeable
395 automated protocols and functional read-out devices can be used within the platform to allow assay
396 flexibility. For each high-throughput application, complex automated, or semi-automated, data
397 analysis is required to identify compounds with either positive or negative phenotypic effect.
398 Identification. Identification of hit compounds is followed by further dose response confirmation.

399

400 **Figure 2**: Interchangeable assay modules can be used with the high-throughput platform

401 The phenotypic screening platform is designed to work with interchangeable read-out modules to
402 allow multiple assays to be carried out on the same platform. The primary output of the platform
403 discussed in this review is the sperm motility assay module (A), that consists of a microscopy-based
404 readout followed by automated sperm tracking and analysis. This provides data on sperm numbers,
405 motility, kinematics, hyperactivation and swimming patterns at multiple timepoints.

406 Further examples of other read-out modules and assays that can be achieved with the high-
407 throughput screening platform are illustrated, including a quantitative acrosome reaction, and
408 sperm cell viability assays using flow cytometry (B), and intracellular calcium quantification using a
409 spectrofluorometer (C). Furthermore, these read-out modules can be multiplexed together to gather
410 additional information within the same automated cycle. For example, this could be a flow
411 cytometry-based assay examining the acrosome reaction or cell viability in response to compounds
412 that follow on from a motility read-out of the same cell population.

413

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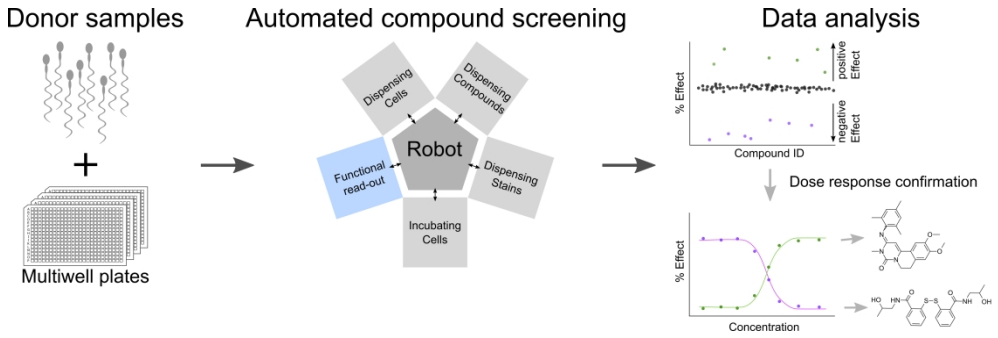

Figure 1: The Flexibility of the high-throughput phenotypic screening platform and automation. The high-throughput sperm screening platform developed by Gruber et al. utilises a 384 well plate format and automation through liquid-handling machines and interchangeable measurement devices. Sperm cells from donor or patients can be observed within the system, and although the focus of platform development has been human sperm, the platform can also be used for the examination animal sperm such as bovine. Depending on the assay format, non-capacitating or capacitating conditions can be used and cells can be exposed to library compounds for defined periods of time, as well as controls and stains appropriate for each application. Interchangeable automated protocols and functional read-out devices can be used within the platform to allow assay flexibility. For each high-throughput application, complex automated, or semi-automated, data analysis is required to identify compounds with either positive or negative phenotypic effect. Identification. Identification of hit compounds is followed by further dose response confirmation.

186x60mm (600 x 600 DPI)

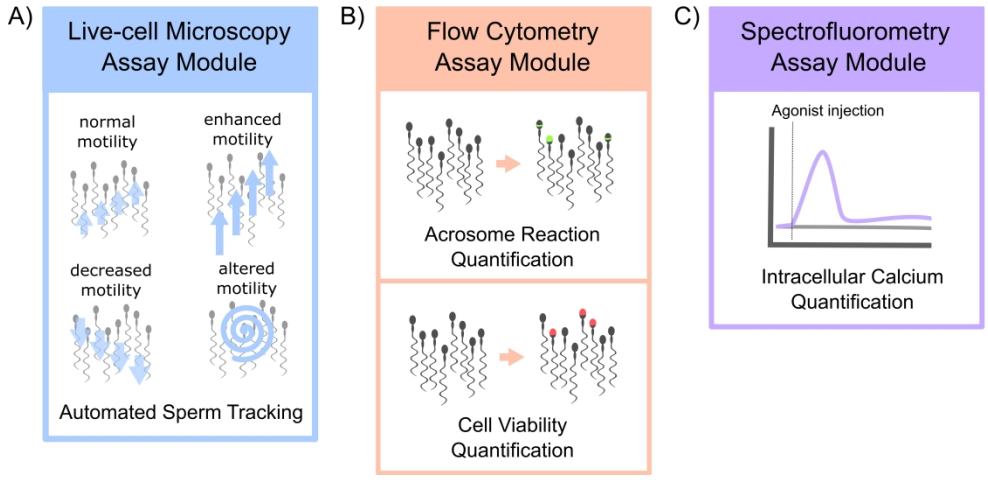

Figure 2: Interchangeable assay modules can be used with the high-throughput platform.
The phenotypic screening platform is designed to work with interchangeable read-out modules to allow multiple assays to be carried out on the same platform. The primary output of the platform discussed in this review is the sperm motility assay module (A), that consists of a microscopy-based readout followed by automated sperm tracking and analysis. This provides data on sperm numbers, motility, kinematics, hyperactivation and swimming patterns at multiple timepoints.
Further examples of other read-out modules and assays that can be achieved with the high-throughput screening platform are illustrated, including a quantitative acrosome reaction, and sperm cell viability assays using flow cytometry (B), and intracellular calcium quantification using a spectrofluorometer (C).
Furthermore, these read-out modules can be multiplexed together to gather additional information within the same automated cycle. For example, this could be a flow cytometry-based assay examining the acrosome reaction or cell viability in response to compounds that follow on from a motility read-out of the same cell population.

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