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Optimization of Sperm Culture Conditions for Human ART

Dual-buffer culture medium delivers improved sperm performance, including sperm viability, motility, and rapid progression

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OVERVIEW

In this study, we hypothesized that a dual-buffer culture medium may help improve sperm performance for assisted reproductive technology (ART). In the following, we demonstrate the superior performance of a commercially-available dual-buffer solution of HEPES and MOPS: Multipurpose Handling Medium-Complete (MHM-C, Catalog #90166, FUJIFILM Irvine Scientific). MHM-C showed significantly better performance, assessed at 8 and 48 hours, for key sperm parameters over single buffer controls: sperm viability, motility percent, and rapid progression MHM-C.

A COMBINATION OF HEPES & MOPS TO MAINTAIN OPTIMUM PH

Sperm performance can be a key differentiating factor in achieving better clinical pregnancy rates. Human sperm handling procedures for ART are commonly performed in room atmosphere, where temperature and CO_2 play a significant role in pH and pH buffering, and differ by as much as 12°C and 5% from typical incubator conditions. Maintaining a precise and stable pH balance is challenging, as even minor environmental fluctuations can damage human sperm.

HEPES and MOPS are zwitterionic organic buffers with pKa at 20°C ~of 7.55 and 7.15 respectively. Individually, they have been extensively studied in biological systems, as well as for sperm washing, oocyte retrieval, embryo transfer, cryopreservation, and ART procedures. However, recent evidence supports the notion that combining HEPES and MOPS may allow for improved media formulations.

MHM-C is a dual-buffer solution of HEPES and MOPS used to maintain sperm, oocytes, and embryos for human ART.

Dual-buffer solutions have demonstrated improvements in working pHe, Na^+ or K^+ concentration, and/or concentration toxicity and osmolality, over single buffer media, and have a pH point.

This study examined if the dual-buffer MHM-C could maintain pH better over a longer period of time during routine sperm handling procedures at room atmosphere, and if sperm that remain in this ideal physiological range longer showed improved performance over controls.

In the following passages, we describe the baseline conditions for human semen samples in three handling media (dual buffer MHM-C compared to two single buffer (HEPES) media: a control Sperm Washing Medium (SPWASH, Catalog #9983), and another commercially-available sperm handling medium (COMP-A) by distribution of motility percent and rapid progression percent. We then quantify causal association of motility percent and rapid progression percent to handling medium after 48 hours at room-atmosphere conditions.

SPERM COLLECTION, PREPARATION, & PROCESSING OF STUDY SAMPLES

Semen samples from 21 subjects were collected and processed as previously described, and diluted to equal concentrations into each sperm processing medium. This study was performed under an institutional review board (IRB), and only specimens that met the World Health Organization 5th Edition criteria for sperm concentration and motility were used.

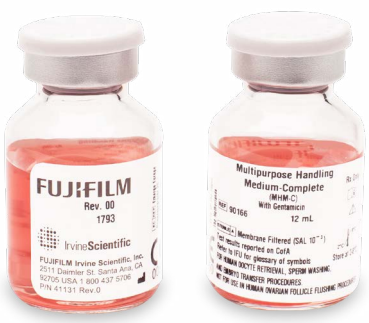

Table 1: Mean and standard deviation (SD) of baseline (0-hour) conditions overall and by medium type

Mean (SD)	MHM-C	COMP-A	SPWASH	Overall
Concentration (mi/ml)	5.03 (0.23)	5.09 (0.28)	5.01 (0.26)	5.04 (0.26)
Motility (%)	63.38 (5.87)	61.14 (7.80)	62.10 (7.73)	62.21 (7.13)
Rapid Progression (%)	48.33 (13.81)	47.81 (12.41)	49.00 (7.06)	48.38 (11.29)

DATA COLLECTION & STATISTICS

Following collection, processing, and preparation of participants' semen samples in the three handling media, the labeled and capped tubes were left in a controlled room temperature environment for 48 hours.

Assessments were performed at 8, 24, and 48 hours, at which times the concentration, motility percent, rapid progression percent, medium progression percent, slow progression percent, and static percent of total sperm were recorded. The mean and standard deviation of the recorded baseline (0-hour) characteristics were calculated to describe the observed location and spread of these variables by handling medium and overall. The distributions of motility percent and rapid progression percent within each handling medium are summarized in box plots at baseline and each follow-up time.

The overall association between motility percent and handling medium was assessed using a linear mixed effects regression model. This model estimates the average motility percent of sperm in each medium, adjusting for the motility percent at baseline and accounting for possible correlation between measurements on the three samples taken from the same individual.

Separate models were used to estimate motility percent at 8 hours and at 48 hours. Analogous linear mixed effects models were fit to assess the association between rapid progression percent and handling medium at 8 hours and at 48 hours.

PRIMARY FINDINGS

Each sample was prepared following the same laboratory protocol and divided into three subsamples, one in each of the study media (MHM-C, COMP-A, SPWASH) for a total of 63 samples.

The study design is balanced across subjects and sperm handling media, and there is no missing data for the analyzed variables. The mean and standard deviation of baseline characteristics of the samples are shown in **Table 1**.

The initial concentration of sperm is very similar across handling media, and there do not appear to be substantial differences in total motility or motility progression categories across the three media.

Improved percent motility in MHM-C over 48 hours

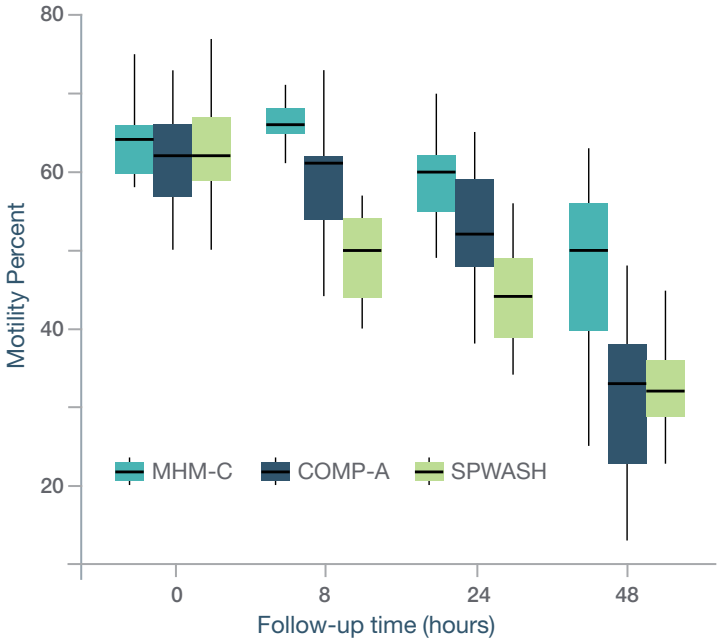

Figure 1. Box plots comparing the distributions of motility percent in MHM-C, COMP-A, and SPWASH at baseline and at 8, 24, and 48 hours. Motility percent appears to be similarly distributed across the three media at baseline, but as time progresses, the motility percent declines more in COMP-A and SPWASH than it does in MHM-C.

Table 2: MHM-C showed statistically significant better mean motility percent compared to COMP-A and SPWASH at 8 hours and 48 hours

Medium	Estimated Difference in Mean Motility	95% Confidence Interval	p Value
COMP-A			
8 H	-7.12	(-10.57, -3.68)	0.000162
48 H	-17.04	(-21.65, -12.44)	3.38e-09
SPWASH			
8 H	-16.60	(-20.04, -13.18)	2.71e-12
48 H	-17.14	(-21.71, -12.57)	2.58e-09

Table 2. The estimated differences in mean motility percent at 8 hours and at 48 hours, comparing samples of sperm preserved in COMP-A to those in MHM-C and comparing samples preserved in SPWASH to those in MHM-C.

MHM-C showed improved percentage of rapid progressive sperm over 48 hours

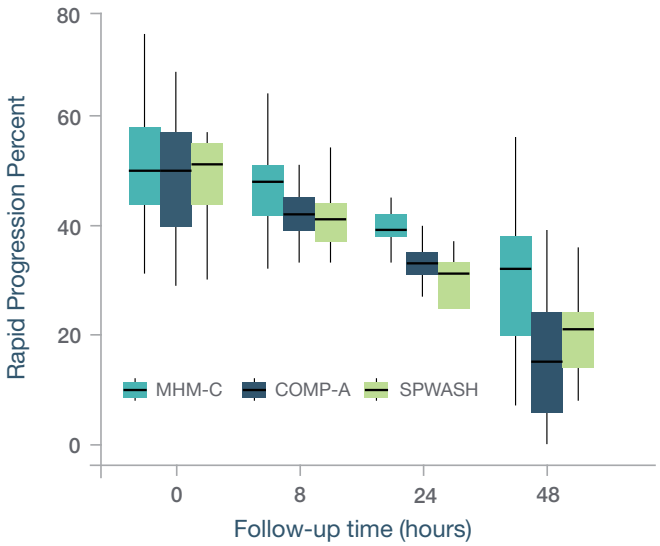

Figure 2. Box plots comparing the distributions of rapid progression percent in MHM-C, COMP-A, and SPWASH at baseline and at 8, 24, and 48 hours. As was observed for overall motility percent, the rapid progression percent appears to be similarly distributed for each of the three media at baseline. At each subsequent follow-up time, the rapid progression percent appears to decline more in COMP-A and SPWASH than it does in MHM-C.

Table 3: MHM-C showed statistically significant better percentage of mean rapid progressive sperm over 48 hours

Medium	Estimated Difference in Mean Motility	95% Confidence Interval	p Value
COMP-A			
8 H	-5.20	(-9.39, -1.00)	0.017
48 H	-13.16	(-18.71, -7.09)	8.53e-05
SPWASH			
8 H	-8.21	(-12.40, -4.02)	0.000321
48 H	-10.60	(-16.09, -5.04)	0.0011

Table 3. The estimated differences in mean rapid progression percent at 8 hours and at 48 hours, comparing samples of sperm preserved in COMP-A to those in MHM-C, and comparing samples preserved in SPWASH to those in MHM-C.

DISCUSSION

Replicating physiological homeostasis minimizes cellular stress during routine sperm handling—generating optimal outcomes for assisted reproductive procedures, such as IUI, ICSI, and IVF.[1]

During incubation, gametes and embryos are readily maintained at ideal temperature, humidity, and CO_2 levels. The CO_2 gas concentration (typically 5–7%) regulates external pH. pHe is easily manipulated and measured; therefore, optimal pHe has been extensively examined for embryo and gamete culture.

However, handling, processing, and preparation steps are typically performed outside of an incubator, where sperm are exposed to atmospheric conditions, and the risk of even minor changes in pHe can lead to undesirable outcomes in sperm viability and performance, and by extension fertility rates. Culture media formulations have been extensively examined to maintain physiologic pHe during routine handling steps performed outside of an incubator and are reviewed in Swain et al. 2011.[2]

Zwitterionic buffers, such as HEPES and MOPS, can act as either an acid or a base and are commonly used to stabilize pHe in cell culture media. It is likely that pHe influences cells in culture through its impact on intracellular pH (pHi) reviewed extensively in Swain 2011.[3] pHi regulates a variety of cellular processes including enzymatic activity, cell division, differentiation, membrane transport, protein synthesis, cell communication, cytoskeleton elements, and microtubule dynamics.[4] Cells contain intrinsic pHi regulatory mechanisms, (HCO_3^-/Cl^- exchanger, Na^+/H^+ antiporter, and Na^+ dependent HCO_3^-/Cl^- exchanger); however in culture, pHi initially follows the external pH of media. Additionally, in culture media, amino acid additives act as metabolic substrates, as well as osmolytes and regulators of pHi.[5] Both taurine and glycine are demonstrated osmolytes and are beneficial for human embryo development.[6,7] Importantly, it has been previously demonstrated that sperm pHi and function are influenced by pHe.[8]

MHM-C combines HEPES and MOPS for an improved formulation that does not require the use of a CO_2 incubator to maintain physiological pH 7.2-7.4 and osmolality over a broad temperature range. Additionally, MHM-C solution also contains glycine and taurine to maintain cellular homeostasis through regulation of pHi.

Human sperm bioassays are highly sensitive when both motility and the quality of sperm motility (motility grade) are taken into consideration.[9] The American Association of Bioanalysts (AAB) chose an assay time of 48 hours for identifying the quality of ART culture media, and this study used 48 hours as an endpoint to be consistent with this guidance, and assayed motility percent and rapid progression percent, to sensitively quantify causal association of sperm performance to handling medium.

The experimental design of this study enabled the causal association between handling medium and rapid and total sperm motility percent. However, it is important to carefully assess whether any unmeasured factors, or factors not analyzed, could explain part or all of the observed differences in motility percent, and the rapid progression percent between the handling media.

Precise handling of cells and supplies within the lab, as well as tedious attention to detail, are just as important as the medium's formulation is to outcomes. We paid careful attention to minimize any differences across media in the handling of samples throughout the experiment and in the measurement tools or procedures used at baseline or during follow-up, so as not to introduce bias into the data.

CONCLUSION

In summary, differences of substantial magnitude and strong statistical significance were observed for mean motility percent between COMP-A and MHM-C, and between SPWASH and MHM-C, at both analyzed follow-up times.

Here, for the first time, superior performance of a commercially-available dual buffer solution of HEPES and MOPS for human sperm handling was demonstrated. Sperm viability, motility percent, and rapid progression in MHM-C displayed significantly better performance than single buffer controls, presumably due to synergistic pHe and pHi stabilization afforded by a dual-buffered system.

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