A Commercially Available Dual-Buffered IVF Handling Medium Containing HEPES and MOPS Maintains Stable pH and Supports Human Sperm Survival, Normal Fertilization Following ICSI and Embryo Development

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INTRODUCTION

Maintenance of a stable media pH is important to reduce environmental stress in the culture environment and optimize assisted reproductive outcomes. Perturbations in pH can disrupt critical intracellular biochemical processes essential for gamete and embryo functioning. For example, oscillations in media pH can alter embryo gene expression, and changes in pH can impact sperm motility and embryo development.

Shifts in media pH are of increased concern for procedures performed outside the laboratory incubator, like cell observation, ICSI, embryo transfer or cryopreservation. Therefore, stabilization of culture media pH during these manipulations helps prevent damaging pH deviations and may improve gamete/embryo quality and ultimately ART success rates.

Zwitterionic buffers, known as Good's Buffers, offer a means by which to increase buffering capacity of culture media and stabilize pH. Current IVF handling media utilize mono-buffered systems, incorporating either the buffer HEPES or MOPS. However, use of a single buffer limits the ability to adjust optimal buffering capacity. For example, the pKa, or optimal buffering of MOPS at 20°C is ~7.20, which may be sufficient for labs that do not warm their culture media or working surfaces, while HEPES has a pKa of ~7.55, which may be too high. However, at 37°C HEPES has a pKa of ~7.31, while MOPS is 6.95. Furthermore, potential toxicity concerns exist with elevated concentrations of buffers used in mono-buffered media. Therefore, traditional handling media utilizing a single buffer may not be ideal. Use of a dual buffered media containing MOPS and HEPES may permit benefits of both buffers and maintain a more appropriate buffering capacity over a range of temperatures, while permitting use of lower buffer concentration.

OBJECTIVE

Examine efficacy of a commercially available handling medium utilizing a combination buffering system of HEPES and MOPS in comparison to HEPES only buffered media by examining pH stability, impact on sperm motility, resulting fertilization rates following ICSI, and embryo development.

MATERIALS & METHODS

pH Stability

-New bottles of HEPES-only buffered media, or a combination HEPES:MOPS media (MHM™, Irvine Scientific) were opened and pH tested weekly over 1 month to compare pH stability. Aliquots of each media were warmed to 37°C and pH measured using a standard bench top pH meter.

24h Human Sperm Motility

-Human sperm were isolated via density gradient centrifugation and resulting samples were split and resuspended, 15-30·10^6/ml with either HEPES-only buffered media or dual-buffered MHM™. Samples were stored at room temperature and sperm motility was examined 24h later. A total of 5 sperm samples were analyzed

96h Mouse Embryo Development

-Frozen 1-cell mouse embryos were thawed and cultured in either a HEPES-only buffered media or the dual-buffered MHM™ for 2h at 37°C in room atmosphere. Control embryos were placed into HTF. Embryos were then placed into HTF media and cultured in 6% CO_2 for 96h. Data were collected over 2 replicates.

ICSI Fertilization Rates

-Following retrieval and denuding, human oocytes were split and placed into either a HEPES-only buffered handling medium or into the dual-buffered MHM™ for sperm injection during ICSI. Presumptive zygotes were then monitored for subsequent fertilization. Data were collected from 47 patients and 594 oocytes.

RESULTS

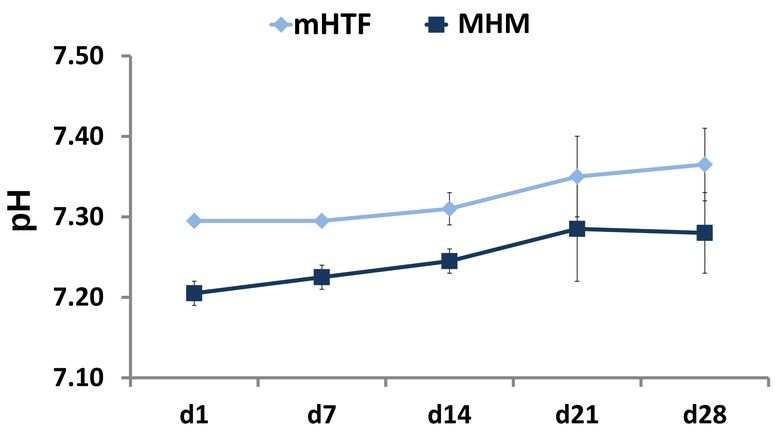

Figure 1: Changes in pH of a HEPES-buffered media and a HEPES:MOPS buffered med (MHM™) over 1 month. pH measurements were taken at weekly intervals.

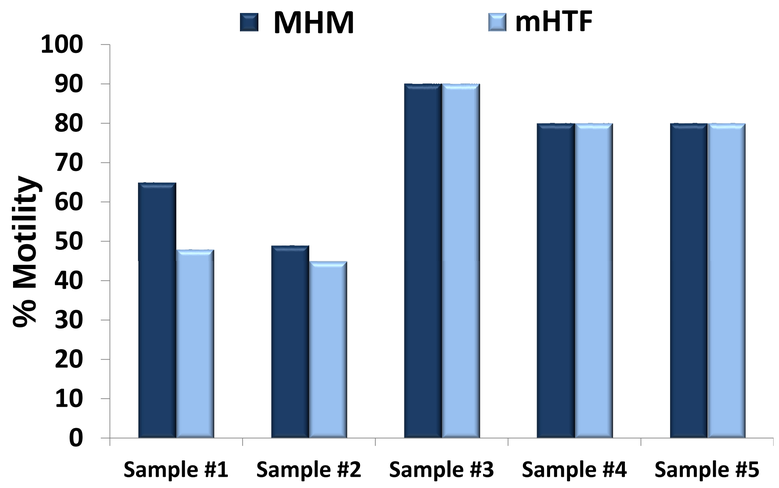

Figure 2: Sperm motility following 24h of culture in HEPES only buffered medium (mHTF) or a medium with HEPES and MOPS (MHM™).

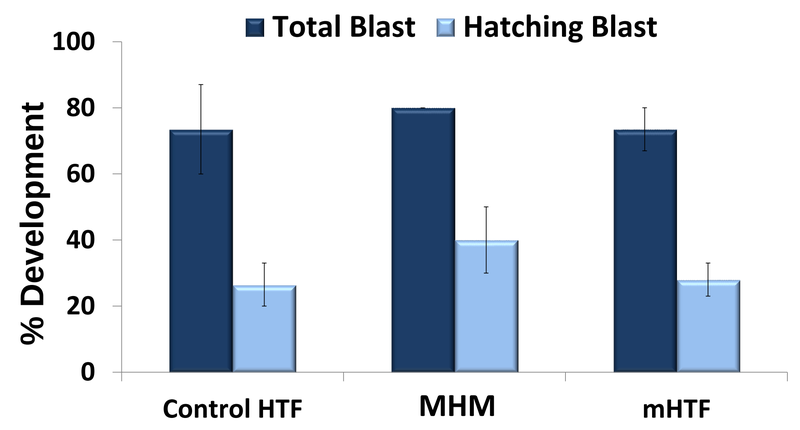

Figure 3: Mouse embryo development following 2h exposure of 1-cell frozen/thawed embryo to various handling media and cultured subsequently for 96h.

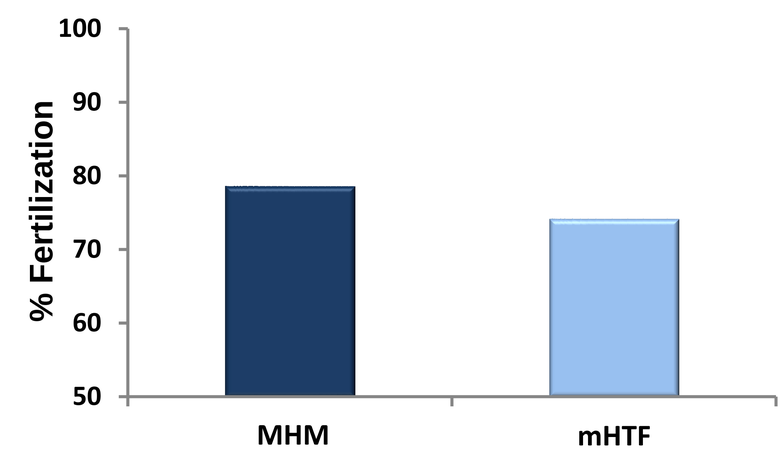

Figure 4: Fertilization of human oocytes following ICSI when held and injected in a HEPES-only buffered medium (mHTF) or a dual buffered media with HEPES and MOPS (MHM™).

CONCLUSIONS

A combination buffer media containing HEPES and MOPS maintains stable pH, supports human sperm motility, mouse embryo development and human oocyte fertilization following ICSI at rates equivalent to HEPES-only buffered media. This approach of combining buffers offers ability to optimize pKa or optimal buffering capacity of IVF handling media.