# Validation of Biorocks C-TYH Medium for Mouse Sperm Capacitation in In-Vitro Fertilization

## Key Findings

- **For robust hybrid strains (B6D2F1):** Standard HTF medium provides excellent and reliable fertilization and blastocyst development rates.
- **For challenging inbred strains (BALB/c):** Using C-TYH for sperm capacitation nearly doubled the 2-cell rate (61.3% vs. 31.0%) and substantially increased the blastocyst rate compared to HTF.
- **For C57BL/6 strains:** C-TYH provided a notable improvement in the 2-cell rate over HTF (83.0% vs. 74.6%), indicating more efficient fertilization.
- **Recommendation:** HTF is the standard choice for robust strains. C-TYH is the recommended medium for improving IVF outcomes with inbred strains (e.g. BALB/c), for troubleshooting low fertilization rates, or when using cryopreserved sperm.

## 1. Introduction

The success of *in-vitro* fertilization (IVF) protocols in murine models is fundamentally dependent on the successful recapitulation of sperm capacitation *in vitro*. While Human Tubal Fluid (HTF) medium is a widely validated, general-purpose medium that consistently produces excellent fertilization results in many applications, certain genetic backgrounds and experimental conditions can benefit from an alternative formulation. To address this, C-TYH is offered as a specialized medium for researchers seeking to optimize capacitation protocols, particularly in cases where HTF may not yield sufficient results. This note provides direct experimental evidence comparing its performance against HTF across several common mouse strains, offering a clear framework for media selection.

## 2. Materials and Methods

**Sperm Collection and Capacitation.** Sperm was collected from male mice of C57BL/6, B6D2F1, and BALB/c strains by making incisions in the cauda epididymis and gently squeezing to release the sperm into a 250 µL drop of C-TYH medium. The sperm suspension was pooled and divided into two experimental groups for capacitation. A capacitation volume of 1.9 mL was used to minimize experimental variables; this larger volume allows sperm from the same male to be split between the two different media groups without significant carryover of the initial collection medium. While this validation was performed using a larger volume for comparative purposes, it is important to note that standard protocols often utilize smaller capacitation volumes (e.g., 200 µL). Based on the internal data we released (**Optimization of Mouse In Vitro Fertilization Protocol for Reduced Media Consumption and Improved Workflow)** and established methodologies from other literatures, using a 200 µL volume of C-TYH for capacitation is expected to yield comparable, successful fertilization outcomes.

- **Group 1 (HTF Capacitation):** A 100 µL aliquot of the initial sperm suspension was transferred into a dish containing 1.9 mL of HTF medium.
- **Group 2 (C-TYH Capacitation):** A 100 µL aliquot of the initial sperm suspension was transferred into a dish containing 1.9 mL of C-TYH medium.

Both groups were incubated for 1 hour in an incubator at 37°C with 5% $CO_2$ and 5% $O_2$ to allow for capacitation.

**In-Vitro Fertilization and Embryo Culture**. Cumulus-oocyte complexes (COCs) were collected from superovulated female mice, pooled, and randomly allocated into fertilization drops prepared under Biorocks mineral oil. For both experimental groups, fertilization was carried out by adding capacitated sperm to 200 µL drops of HTF medium containing the COCs. For the C57BL/6 and B6D2F1 strains, 10 µL of capacitated sperm was added. For the BALB/c strain, this volume was increased to 30 µL to compensate for an observed lower sperm concentration. The fertilization dishes were incubated for 4-5 hours. Following fertilization, presumptive zygotes were washed and transferred to Biorocks KSOM for culture. Embryo development was assessed on Day 1 for cleavage to the 2-cell stage and on Day 4 for development to the blastocyst stage.

### 3. Results and Discussion

To empirically validate the performance of C-TYH against the established HTF standard, a comparative IVF study was conducted using the three distinct mouse strains. The subsequent development of oocytes to the 2-cell and blastocyst stages was recorded.

**Table 1: Comparative IVF Outcomes using HTF and C-TYH for Capacitation**

| Strain | Capacitation Medium | Number of D0 oocytes | Number of D1 2 cells | Number of D4 Blastocysts | 2 cells Rate | Oocytes to Blastocyst Rate | 2 cells to Blastocyst Rate |
|---|---|---|---|---|---|---|---|
| C57BL/6 | HTF | 59 | 44 | 24 | 74.6% | 40.7% | 54.5% |
| | C-TYH | 53 | 44 | 23 | 83.0% | 43.4% | 52.3% |
| B6D2F1 | HTF | 89 | 74 | 63 | 83.1% | 70.8% | 85.1% |
| | C-TYH | 90 | 73 | 69 | 81.1% | 76.7% | 94.5% |
| BALB/C | HTF | 29 | 9 | 7 | 31.0% | 24.1% | 77.8% |
| | C-TYH | 31 | 19 | 13 | 61.3% | 41.9% | 68.4% |

The results demonstrate a clear, strain-dependent difference in performance between the two media.

- For the **B6D2F1** hybrid strain, both HTF and C-TYH produced excellent 2-cell and blastocyst rates, confirming the utility of HTF as a high-performance medium for robust strains.
- For the **C57BL/6** inbred strain, C-TYH provided a notable improvement in the 2-cell rate (83.0% vs. 74.6%), indicating more efficient fertilization.

- The most significant difference was observed with the **BALB/c** inbred strain. C-TYH nearly doubled the 2-cell rate (61.3% vs. 31.0%) and substantially increased the oocytes to blastocyst rate (41.9% vs. 24.1%) compared to HTF. This result suggests that BALB/c sperm capacitate far more effectively in C-TYH.

**4. Strategic Application and Recommendations**

Based on the experimental evidence, the following application strategy is recommended:

- HTF remains the recommended medium for routine IVF protocols utilizing robust hybrid mouse strains (e.g., B6D2F1), where it demonstrates consistently high fertilization and development rates.
- C-TYH is strongly recommended as the medium of choice for:
    - Protocols involving inbred strains, particularly when suboptimal results are observed with HTF. The data from C57BL/6 and BALB/c provide direct evidence of its superior performance.
    - Troubleshooting protocols that yield low or inconsistent fertilization rates.
    - Experiments utilizing cryopreserved sperm, which may benefit from the C-TYH formulation.

**5. Conclusion**

While HTF is an effective and reliable medium for many routine mouse IVF procedures, C-TYH represents a validated, high-performance alternative engineered to meet the more stringent requirements of sensitive murine models. The data clearly show that for challenging inbred strains like BALB/c and C57BL/6, capacitation in C-TYH leads to significantly improved fertilization and development rates. This targeted approach to media selection is essential for maximizing experimental reproducibility and success across a wider range of applications.