Optimization of Mouse In Vitro Fertilization Protocol for Reduced Media Consumption and Improved Workflow

Key Findings

- **HTF Volume Can Be Reduced:** The volume of Biorocks HTF medium used for IVF can be reduced by up to 90% without a statistically significant drop in embryo development rates for the KM mouse strain.
- **Culture with HTF to 2-cells Stage is Viable but Carries Risks:** A simplified protocol that skips the post-fertilization wash step and involves overnight co-culture of embryos and sperm in HTF (Method 3) performed well with the KM strain.
- **KSOM Remains the Gold Standard for Post-IVF Culture:** Despite the success of the simplified method in this specific experiment, established best practices strongly support washing embryos and transferring them to a specialized medium like Biorocks KSOM. This is crucial to avoid toxicity from cellular debris and to help embryos overcome the 2-cell block, a common issue in many mouse strains.
- **Optimal Recommended Protocol:** Method 1, which reduces Biorocks HTF volume by 75% but maintains the standard practice of washing and transferring embryos to Biorocks KSOM, is recommended as the best balance of efficiency and safety for broad application across different mouse strains.

1. Introduction 引言

The *in vitro* Culture of preimplantation mouse embryos is a fundamental technique underpinning advances in developmental biology, genetics, and assisted reproductive technologies [1]. The success of *in vitro* fertilization (IVF) is critically dependent on the composition and handling of the Culture media. Our laboratory has previously established a standard operating procedure (SOP) utilizing Biorocks Human Tubal Fluid (HTF) for fertilization and Biorocks Potassium Simplex Optimized Medium (KSOM) for post-fertilization Culture, a system designed to provide high rates of embryonic development.

In response to user feedback, the present study was initiated to investigate strategies for optimizing this SOP. The objectives were twofold: first, to validate protocols that minimize HTF consumption, and second, to evaluate a procedural modification aimed at simplifying the post-fertilization workflow.

2. Materials and Methods

2.1. Animal Models and Gamete Collection

All experiments utilized the outbred mouse strain KM, selected for its characteristically high oocyte yield. Fresh sperm was collected from two fertile males for this study. Cumulus-oocyte complexes (COCs) were retrieved from superovulated females and pooled to ensure random, homogenous allocation across experimental groups, thereby eliminating gamete quality as a confounding variable.

2.2. Experimental Design and IVF Procedure

Four IVF protocols were compared: a Baseline method and three modified methods.

- **Sperm Capacitation:** A pooled sperm sample was prepared in 400 µL of HTF. This suspension was then divided. For the Baseline, Method 1, and Method 3 groups, 200 µL of the suspension was transferred to 1.8 mL of fresh HTF, creating a 2 mL capacitation volume dish. For the Method 2 group, the remaining 200 µL of the original suspension was used directly for capacitation. All capacitation dishes and drops were overlaid with Biorocks mineral oil. Capacitation proceeded for 1 hour at 37°C, 5% CO_2, and 5% O_2.
- **Fertilization:** Pooled COCs were randomly allocated to the different fertilization dishes/drops to eliminate any potential variance in oocyte quality between groups. Fertilization was conducted at a density of ~60 COCs per vessel. To maintain a consistent final sperm concentration across groups, insemination was performed as follows:
    - **Baseline Group:** Fertilization was conducted in a 2 mL dish of HTF, inseminated with 100 µL of capacitated sperm .
    - **Method 1 & 3 Groups:** Fertilization was conducted in a 200 µL drop of HTF, inseminated with 10 µL of capacitated sperm.
    - **Method 2 Group:** Fertilization was conducted in a 200 µL drop of HTF, inseminated with 1 µL of capacitated sperm.

2.3. Embryo Culture and Developmental Assessment

- **Baseline, Method 1, Method 2:** Following a 4-5 hour co-incubation, presumptive zygotes were washed through four successive 300 µL drops of HTF, followed by a single 300 µL drop of KSOM.
- **Method 3:** Presumptive zygotes were left undisturbed in the fertilization drop overnight. On Day 1, embryos that had cleaved to the 2-cell stage were washed in KSOM.

All embryos were subsequently Cultured in 30 µL drops of KSOM under mineral oil at 37°C, 5% CO_2, and 5% O_2. Developmental progress was assessed at Day 1 (cleavage to 2-cell stage) and Day 4 (formation of blastocyst).

3. Results

The developmental rates for each method are presented in Table 1. While minor numerical variations were observed, the results show no statistically significant differences in fertilization rates or subsequent developmental competence to the blastocyst stage between the Baseline and any of the modified methods. All four protocols yielded robust developmental outcomes.

Table 1: Comparative Developmental Outcomes of IVF Protocols

	Number of D0 oocytes	Number of D1 2 cells	Number of D4 Blastocysts	2 cells Rate	Oocytes to Blastocysts Rate	2 cells to Blastocysts Rate
Baseline	261	183	102	70.1%	39.1%	55.7%
Method 1	274	176	102	64.2%	37.2%	58.0%
Method 2	278	191	109	68.7%	39.2%	57.1%
Method 3	169	124	78	73.4%	46.2%	62.9%

4. Discussion

The primary objective of this study was to validate methods for reducing HTF consumption. The tested protocols offer significant media savings compared to the baseline, as calculated for a standard experiment involving 2 males and 10 females (Table 2).

Table 2: Comparative HTF Volume Usage and Savings Per Experiment (2 males and 10 females)

Method	Capacitation Vol.	Fertilization Vol.	Total HTF Volume	% Reduction
Baseline	4 mL (2 mL x 2 males)	20 mL (2 mL x 10 females)	24 mL	0%
Method 1	4 mL (2 mL x 2 males)	2 mL (0.2 mL x 10 females)	6 mL	75%
Method 2	0.4 mL (0.2 mL x 2 males)	2 mL (0.2 mL x 10 females)	2.4 mL	90%

Having established the clear resource management benefits, the critical question was whether these reductions impacted biological outcomes. This study confirms that mouse IVF protocols can be modified to improve efficiency without a statistically significant loss of performance. The comparable outcome of Method 3 further suggests that for a outbred strain like KM, the immediate culture environment may be more forgiving.

However, the viability of Method 3 must be interpreted with caution. A key purpose of the standard post-fertilization wash step is to remove embryos from an environment that, after several hours, contains dying sperm and cellular debris that release cytotoxic reactive oxygen species (ROS). Furthermore, the protocol delays the introduction of a medium specifically designed to overcome the 2-cell block. This developmental checkpoint is a major hurdle for many mouse strains, particularly inbred lines, when Cultured *in vitro* [3]. Fertilization media like HTF are formulated to support gamete viability and fertilization, not to provide the specific metabolic and ionic requirements needed for the zygote-to-blastocyst transition. In contrast, KSOM was specifically designed with low glucose and optimized potassium concentrations precisely to overcome this block [2, 3]. This principle is underscored in authoritative texts such as *Manipulating the Mouse Embryo: A Laboratory Manual*, which strongly recommends transferring zygotes to an amino-acid supplemented KSOM for all strains, especially those known to exhibit a 2-cell block, rather than continuing Culture in HTF [1].

While the KM strain embryos in this study successfully navigated the first cleavage in HTF, this result should not be broadly extrapolated. For more sensitive inbred strains, or for experiments where maximizing the yield from valuable or genetically modified animals is paramount, prolonged Culture in a non-optimal medium like HTF introduces an unnecessary risk of developmental failure. The safest and most scientifically sound approach is to move zygotes into a developmentally appropriate medium as soon as is practical. The standard procedure of washing zygotes and transferring them to KSOM 4-5 hours post-insemination remains the gold standard for ensuring the highest probability of success across the widest range of genetic backgrounds.

5. Conclusion

This study validates that reducing HTF volume is an effective and safe method for improving the cost-efficiency of murine IVF. While a simplified protocol involving overnight Culture in HTF (Method 3) was not detrimental for the KM strain used here. Given the critical role of specialized media in overcoming the 2-cell block in many mouse strains, we continue to recommend the transition from a fertilization medium (HTF) to a post-fertilization Culture medium (KSOM) as a crucial step. Therefore, Method 1, which combines significant media savings with the established safety of an early transition to KSOM, represents the optimal, validated protocol for broad application.

6. References

[1] Behringer, R., et al. (2014). *Manipulating the Mouse Embryo: A Laboratory Manual*. Cold Spring Harbor Laboratory Press.

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