Performance Validation of Biorocks ART Media (KSOM, HTF, and Mineral Oil) for the In Vitro Culture of Mouse Embryos

1. Introduction

The *in vitro* culture of preimplantation mouse embryos is a fundamental technique underpinning advances in developmental biology, genetics, and assisted reproductive technologies (ART). The composition of the culture medium is arguably the most critical variable influencing experimental outcomes, directly impacting cleavage rates, blastocyst formation, and subsequent embryo viability. Potassium Simplex Optimized Medium (KSOM) was specifically formulated to support the nutritional and metabolic requirements of early-stage embryos, notably overcoming the 2-cell block, a phenomenon of developmental arrest frequently observed in embryos from many inbred and outbred strains cultured *in vitro*.

While KSOM is the primary growth medium, the success of ART procedures, particularly *in vitro* fertilization (IVF), also depends critically on the quality of ancillary reagents. Human Tubal Fluid (HTF) medium is essential for gamete handling, capacitation, and fertilization, while high-purity mineral oil is required to overlay the microdrops, preventing evaporation and maintaining stable pH and osmolarity. The performance of these components can vary between commercial suppliers, necessitating rigorous validation to ensure experimental reproducibility.

This study presents a comprehensive performance validation of the Biorocks ART media suite. Our objective was to evaluate the capacity of Biorocks KSOM, Biorocks HTF, and Biorocks Mineral Oil to collectively support preimplantation development across four different mouse strains (B6D2F1, ICR, KM, and C57BL/6) using embryos derived from both natural mating and IVF.

2. Materials and Methods

2.1. Animal Models

Female (6-8 weeks old) and male mice from four strains were utilized: B6D2F1 (F1 hybrid), ICR (outbred), KM (outbred), and C57BL/6 (inbred). All animals were housed under standard laboratory conditions with a 12-hour light/dark cycle and unlimited access to food and water. All procedures were conducted in accordance with guidelines for animal care and use.

2.2. Embryo Procurement

A standard superovulation regimen, consisting of an intraperitoneal injection of Pregnant Mare Serum Gonadotropin (PMSG) (5IU) followed 48 hours later by human Chorionic Gonadotropin (hCG) (5IU), was administered to all female mice prior to embryo procurement.

2.2.1. Natural Mating

Following hCG injection, superovulated females were paired with fertile males of the same strain. The morning after coitus (Day 0.5), females were examined for the presence of a copulatory plug. Presumptive zygotes were retrieved by flushing the oviducts of plug-positive females.

2.2.2. In Vitro Fertilization (IVF)

For IVF, gametes were prepared and co-incubated following a defined protocol. Sperm were collected from the cauda epididymis and vas deferens of fertile males and released into a 35 mm dish containing 2 mL of Biorocks HTF medium, overlaid with Biorocks Mineral Oil. The sperm were allowed to capacitate for at least one hour in a tri-gas incubator (37°C, 5% CO_2, 5% O_2). Following the capacitation period, Cumulus-Oocyte Complexes (COCs) were retrieved from the oviducts of superovulated females and placed into a separate fertilization dish, also containing 2 mL of Biorocks HTF under Biorocks Mineral Oil. Fertilization was initiated by adding 100 µL of the capacitated sperm suspension to the dish containing the COCs and co-incubating the gametes for 4-5 hours. Following co-incubation, presumptive zygotes were subjected to a multi-step washing protocol. Embryos were first washed in a dish containing 2 mL of fresh Biorocks HTF, followed by sequential washing through four separate 300 µL drops of Biorocks HTF. Finally, the embryos were washed three times in a 300 µL drop of Biorocks KSOM before being transferred into the final culture microdrops.

2.3. Embryo Culture and Developmental Assessment

All presumptive zygotes, regardless of origin, were cultured in Biorocks KSOM. Embryos were group-cultured (20-30 embryos/drop) in 30 µL drops of medium under Biorocks Mineral Oil. Culture was maintained for 4 days in an incubator at 37°C with 5% CO_2 and 5% O_2.

Developmental progress was morphologically assessed at two primary endpoints: cleavage to the 2-cell stage (by Day 1) and formation of a blastocyst, characterized by a distinct inner cell mass and blastocoel, by Day 4. Representative images were captured at key stages.

2.4. Data Analysis

Developmental rates were calculated as percentages based on the total number of presumptive zygotes cultured. The conversion rate from the 2-cell stage to the blastocyst stage was also calculated to assess post-cleavage developmental competence.

3. Results

3.1. Overall Developmental Competence in Biorocks KSOM

The Biorocks ART media suite successfully supported the in vitro development of embryos from all four strains and both procurement methods. High rates of cleavage to the 2-cell stage and subsequent development to morphologically normal blastocysts were consistently observed, as detailed in Table 1.

Table 1 In Vitro Development of Mouse Embryos from Different Strains in Biorocks KSOM

	B6D2F1 (Mating)	B6D2F1 (IVF)	ICR (Mating)	ICR (IVF)	KM (Mating)	KM (IVF)	C57BL/6 (Mating)	C57BL/6 (IVF)
Number of Presumptive Zygotes	1117	651	445	395	2368	1032	173	170

2 cells	867	595	383	330	1605	897	90	139
Blastocyst (Day 4)	798	554	305	264	1093	551	71	118
Presumptive Zygotes to 2 cells rate	77.6%	91.4%	86.1%	83.5%	67.8%	86.9%	52.0%	81.8%
Presumptive Zygotes to Blastocysts rate	71.4%	85.1%	68.5%	66.8%	46.2%	53.4%	41.0%	69.4%
2 cells to Blastocysts rate	92.0%	93.1%	79.6%	80.0%	68.1%	61.4%	78.9%	84.9%

3.2. Comparison of Embryos from Natural Mating vs. IVF

A notable finding was the consistently superior performance of embryos derived from IVF across multiple strains. For the B6D2F1 strain, the IVF-derived cohort yielded a blastocyst rate of 85.1%, significantly higher than the 71.4% from the mated cohort. A similar, even more pronounced trend was observed for the C57BL/6 inbred strain, where the blastocyst rate jumped from 41.0% (Mating) to 69.4% (IVF). The KM strain also showed improvement, with the blastocyst rate increasing from 46.2% (Mating) to 53.4% (IVF). For the ICR strain, performance was robust and comparable between the two methods (~67-68% blastocyst rate).

3.3. Strain-Specific Developmental Rates

The B6D2F1 hybrid strain demonstrated the highest developmental potential, as expected. Critically, the conversion rate from the 2-cell to the blastocyst stage for B6D2F1 embryos was outstanding, at 92.0% (Mating) and 93.1% (IVF). The outbred ICR strain also showed strong development, with approximately 80% of 2-cell embryos progressing to blastocysts. The inbred C57BL/6 and outbred KM strains proved to be the most challenging models, exhibiting lower overall blastocyst rates, particularly from natural mating. However, both strains showed marked improvement with IVF, and their 2-cell to blastocyst conversion efficiency remained high (C57BL/6: ~79-85%; KM: ~61-68%), indicating that the medium supports post-cleavage development even in sensitive strains.

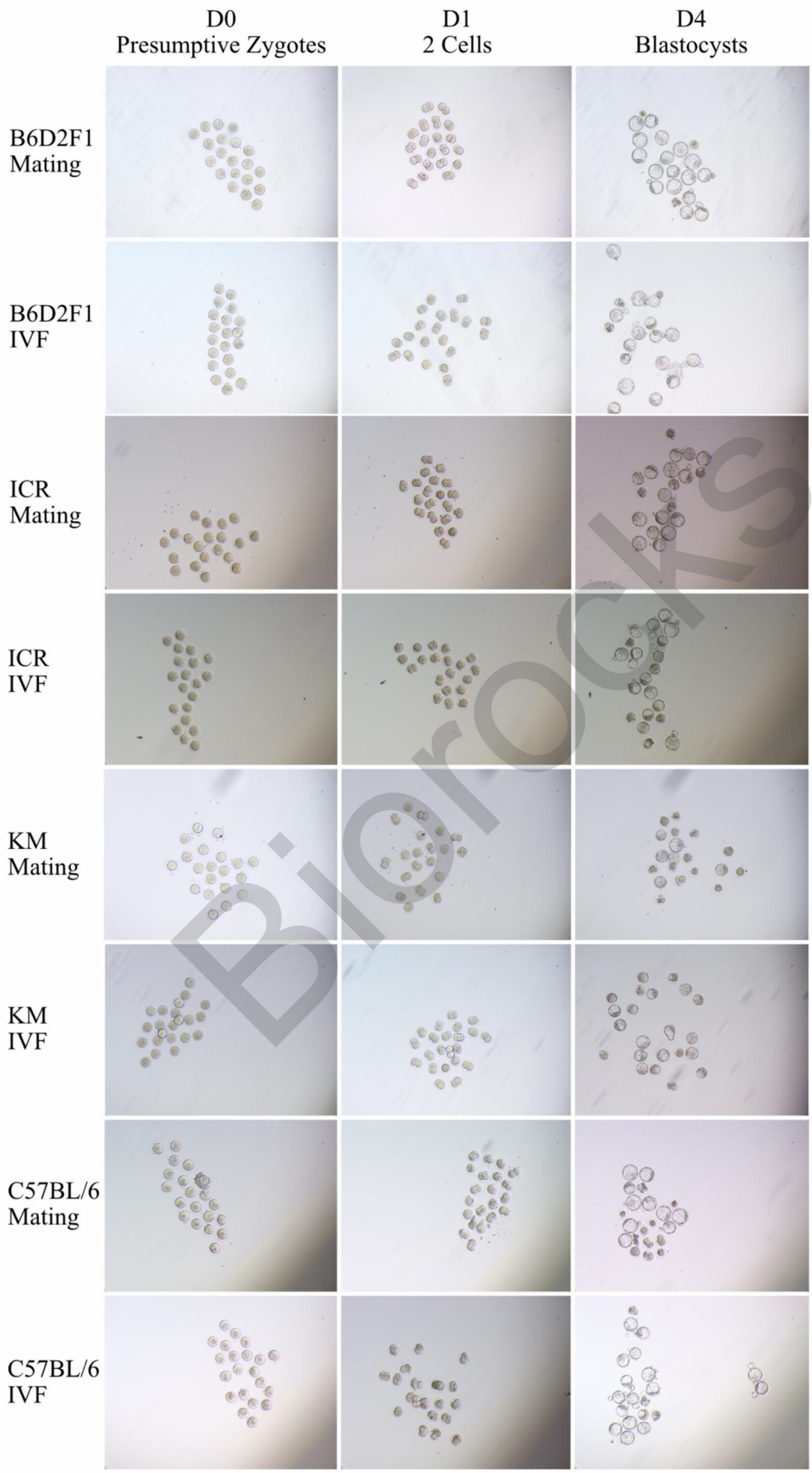
D0
Presumptive Zygotes
D1
2 Cells
D4
Blastocysts
B6D2F1
Mating
B6D2F1
IVF
ICR
Mating
ICR
IVF
KM
Mating
KM
IVF
C57BL/6
Mating
C57BL/6
IVF

Figure 1. Representative images of mouse embryo development in Biorocks KSOM and mineral oil, showing healthy development from the zygote to the blastocyst stage.

4. Discussion

This study provides a comprehensive validation of the Biorocks ART media suite, demonstrating its capacity to support robust preimplantation development across multiple variables. The data confirms the efficacy of the integrated system and highlights its versatility for modern research applications.

While the primary growth medium is KSOM, the successful outcomes are also a testament to the quality of the Biorocks HTF and Mineral Oil. The high fertilization rates achieved in the IVF procedures indicate that Biorocks HTF effectively supports gamete viability, sperm capacitation, and fertilization. Furthermore, the consistent development of embryos in microdrop culture over four days demonstrates that Biorocks Mineral Oil is of high purity, is non-toxic, and effectively protects the delicate microenvironment from evaporation and deleterious shifts in pH and osmolarity.

The superior performance of IVF-derived embryos, particularly for the B6D2F1, KM, and C57BL/6 strains, is a significant finding. This suggests that the Biorocks media system provides an optimal environment that can capitalize on the advantages of IVF, such as precise fertilization timing and the potential for selecting higher-quality gametes, leading to a more synchronous and viable cohort of zygotes.

Analysis of the "2-cell to blastocyst" conversion rate offers deeper insight into the medium's function. The exceptionally high conversion rates (>92%) for the vigorous B6D2F1 strain indicate that the nutrient and energy substrate composition of Biorocks KSOM is not a limiting factor for post-cleavage development. For more challenging strains like KM, while the overall blastocyst rate is lower, the medium still facilitates development, suggesting that initial zygote quality may be the primary determinant of success for those strains.

The consistent performance across genetically diverse strains—a vigorous hybrid (B6D2F1), two different outbred stocks (ICR, KM), and a widely used inbred strain (C57BL/6)—underscores the formulation's robustness. This versatility is highly valuable, as it minimizes the need for media optimization between different projects or mouse models, thereby enhancing experimental consistency and efficiency.

5. Conclusion

In conclusion, the Biorocks ART media suite, including KSOM, HTF, and Mineral Oil, provides a robust and versatile system that consistently supports high rates of preimplantation development for mouse embryos from diverse genetic and procurement origins. Its demonstrated efficacy for both in vivo- and in vitro-fertilized embryos validates its suitability as a high-performance, all-purpose media system for a wide array of applications in reproductive biology and transgenic research.