FACULTY OF HEALTH AND MEDICAL SCIENCES
UNIVERSITY OF COPENHAGEN

EMBRYOTRANS BIOTECH

New IVF Media affect Blastocyst Development and Gene Expression Levels in *in vitro* Produced Bovine Embryos

J. M. K. Nielsen*[1], C. Wrenzycki[2], P. Hyttel[1], F. Poppicht[2], L. Strøbech[3]
Department of Veterinary Clinical and Animal Sciences, University of Copenhagen, Copenhagen, Denmark;[1], Clinic for Veterinary Obstetrics, Gynecology and Andrology, Giessen, Germany;[2], EmbryoTrans Biotech ApS, Frederiksberg, Denmark[3]

Introduction

Most media used for *in vitro* production of bovine embryos have to be supplemented with various compounds prior to use in the IVF laboratory. This is time-consuming and increases the risk of inconsistent media batches.

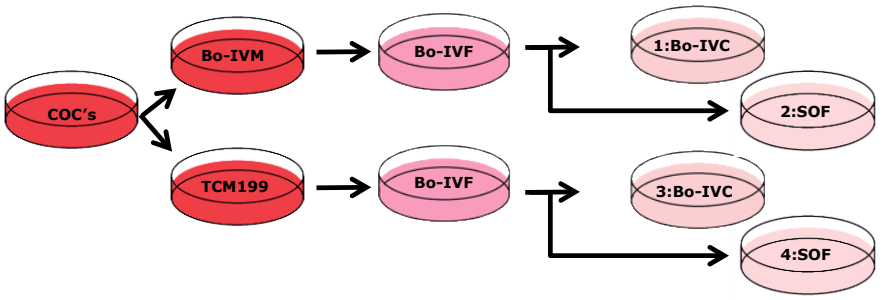
We have tested a novel *in vitro* maturation (IVM), Bo-IVM and *in vitro* culture (IVC), Bo-IVC media (two new commercially available "Ready To Use" media from EmbryoTrans Biotech ApS (Denmark)) vs. standard IVM and IVC media TCM199 and SOF, respectively, from GmBH Minitüb (Germany).
The performance of the media were evaluated as:
-Embryo development as blastocyst rates, morphology and kinetics.
-Gene expression levels of 8 genes associated with critical processes and developmental competence of the embryo

Materials and Methods

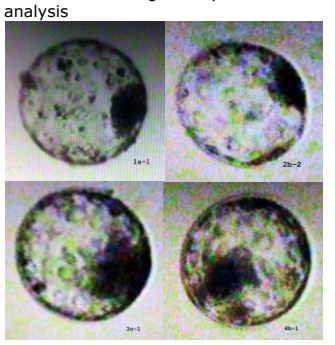
Maturation (IVM)
Cumulus oocyte complexes aspirated from slaughterhouse ovaries were randomized in two groups and matured for 22 hours in either Bo-IVM or TCM199 (+0.5% BSA,+10IU PMSG, +5IU HCG)
(T:38.8, 5.5% CO_2, 21%O_2)

Fertilization (IVF)
Oocyte maturation was evaluated as expansion of cumulus cells. For fertilization both groups were treated equally with media from EmbryoTrans Biotech. Semen was washed in Bo-SemenPrep, and all oocytes fertilized in Bo-IVF medium for min. 19 hours
(T:38.8, 5.5% CO_2, 21%O_2)

Culture (IVC)
The presumptive zygotes were randomized to culture in either Bo-IVC or SOF(+0.5%BSA) for 7 days
(T:38.8, 6% CO_2, 6%O_2, 88%N_2)



Blastocyst Development, Kinetics and Morphology
On day 8 post fertilization, blastocyst rates, morphology and kinetic scores were evaluated. High quality, non-hatched blastocyst from each group were selected for gene expression analysis

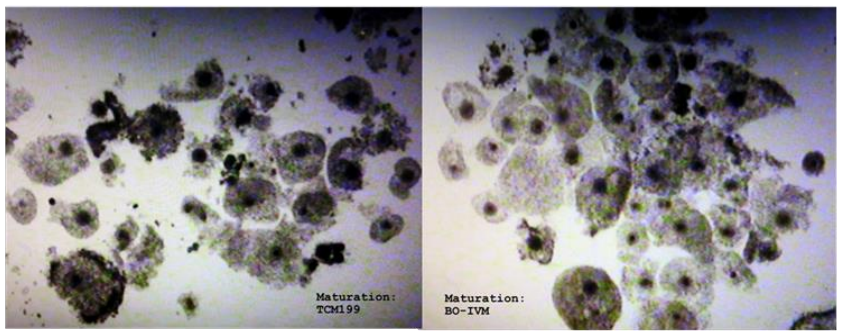


Blastocyst: Gene Expression Analysis
Gene expression analysis was performed on single blastocysts by the RT-qPCR technique using earlier verified primers of the genes; BAX, BCL2L1, DNMT3a, FASN, G6PD, HSPA1A, SLC2A1 and SLC2A3. MessengerRNA (mRNA) from rabbit globin was used as an external control. Isolation of mRNA was done by the use of the Dynabeads® mRNA Direct™ Micro Kit (Invitrogen, Norway). RT reaction was performed in a total volume of 20µl using 2.5 µM random hexamers and a RT reaction mix containing 1x PCR buffer, 5 mM MgCl, 1 mM dNTP's, 20 U/µl RNase inhibitor and 50U/µl MuLV reverse transcriptase. RT reaction was carried out at 25°C for 10 min, 1 hour at 42°C, and 5 min at 99°C, followed by a flash cooling on ice.
Quantitative PCR was carried out in a C1000™ Thermal Cycler (BioRad, Hercules, CA, USA), using 10µl IQ™ SYBR® Green Supermix, 0.2µM forward primer and 0.2µM reverse primer for each gene of interest and the external reference gene. cDNA from each sample was added in embryo equivalents varying from 0.025 to 0.2. Water was added to a total volume of 20 µL. The PCR protocol was as follows: 10 min at 95°C followed by 43 cycles with 15 sec at 95°C, 30 sec at 60°C and 30 sec at 72°C
The relative abundance was calculated using the external control for normalization and the ΔΔCt approach. The efficiency range between the target and the endogenous control amplifications lay between 95 and 100%.

Results

Oocyte Maturation

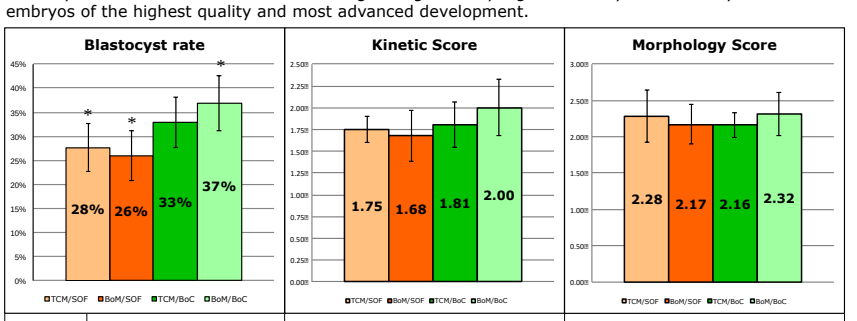
Cumulus oocyte complexes matured in Bo-IVM in general displayed more abundant cumulus expansion and viscoelasticity than their counterparts matured in TCM199.



Difference in morphology between the two maturation groups, Left: oocytes matured in TCM199. Right: oocytes matured in Bo-IVM.

Blastocyst Development, Kinetics and Morphology

Blastocysts cultured in the Bo-IVC medium gave significantly higher blastocyst rates and yielded more embryos of the highest quality and most advanced development.

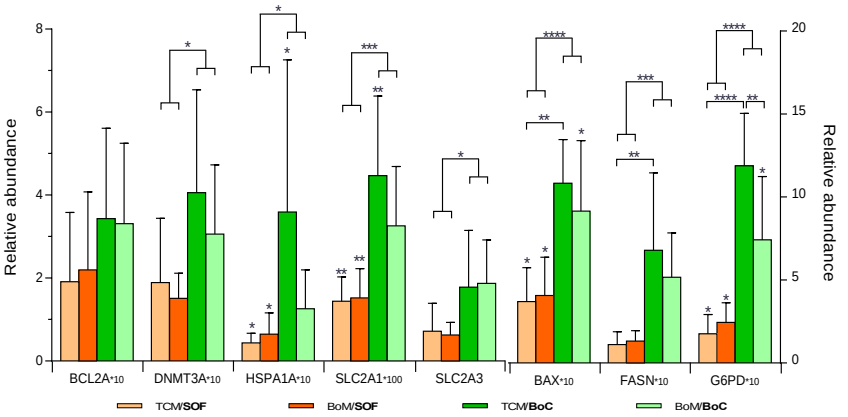


Group (IVM/IVC)	N	Bl.	Bl. Rate (%)	Ha	Exp	n-Exp	Total score	Exc	Good	Poor	Total score
TCM/SOF	308	85	28 ± 2.49	5	54	26	1.75±0.15	42	25	18	2.28±0.36
BoM/SOF	273	71	26 ± 7.16	4	40	27	1.68±0.29	27	29	15	2.17±0.27
TCM/BoC	301	99	33 ± 5.36	20	40	39	1.81±0.26	42	31	26	2.16±0.17
BoM/BoC	274	101	37 ± 7.54	32	37	32	2.00±0.32	56	21	24	2.32±0.30

Blastocyst Development, Kinetic and Morphology scores
Summarized results from all four replicates, showing mean values and standard deviations of blastocyst rates, kinetic and morphology scores. IVM media: TCM199 (TCM), Bo-IVM (BoM). IVC media: SOF (SOF), Bo-IVC (BoC). Significant differences ($p<0.01$) between groups are indicated by * above columns. Error bars in figures: left; 95% confidence interval, middle and right; standard deviations.
N: number of presumptive zygotes, Bl.: Blastocysts:, Bl.Rate: Blastocyst rate, Ha: Hatched, Exp: expanded, n-Exp: non-expanded, Exc: excellent.

Gene Expression Levels

The Bo-IVC medium significantly altered gene expression levels of most genes tested. A two way ANOVA revealed a significant effect of culture media on 7 out of 8 genes.



Relative abundance of mRNA measured in single blastocysts
Mean values and standard deviations of the relative abundance of mRNA, measured in single blastocysts. IVM media: TCM199 (TCM) and Bo-IVM (BoM). IVC media: SOF(SOF) and Bo-IVC(BoC). #of tested blastocysts pr. group: TCM/SOF (n=6), BoM/SOF (n=7), TCM/BoC (n=6), BoM/BoC (n=6). Significant differences between groups are indicated by * above columns, where **** = $p \leq 0.0001$, *** = $p \leq 0.001$, **= $p \leq 0.01$, * = $p \leq 0.05$. Asterix in the top of the figure indicates significant differences between culture media.

Conclusion

The combination of Ready to Use Bo-IVM and Bo-IVC media increased blastocyst rates, kinetic and morphology scores compared to blastocysts produced in a combination of TCM199 and SOF.
Blastocyst cultured in Bo-IVC medium had an increased gene expression, compared to blastocysts cultured in SOF medium.

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