ARE BLASTOCYST RATES A RELIABLE INDICATOR FOR THE QUALITY OF AN IVP SYSTEM?

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Aim: Usually the quality and efficiency of an IVP system is estimated by the blastocyst rates. To obtain more complete information we have in addition to blastocyst rates included morphology and kinetics in the evaluation. In this study we show how oxygen tension and co-culture can modulate embryo quality and kinetics subsequent to IVC in the same medium (SOF), even though the blastocyst rates are very similar. We propose that the following equation can be used as a tool to assess the overall relative efficiency of an IVP system: **BL x M x K** where **BL** is the total blastocyst rate, **M** the mean morphology score, and **K** the mean developmental stage score.

Results and Conclusions:

Table 1: By use of the **BL x M x K** equation it was possible to rank the four IVC groups according to overall in-vitro performance. The highest (best) SOF-IVC score was obtained in 5% O_2 (score 155), closely followed by IVC in 20% O_2 /BOEC (score 143). The lowest scores were obtained after IVC in 5% O_2 /BOEC (score 117) and in 20% O_2 (score 85).

Table 2: Regardless of the BL stage (XB or H) or the culture system, grade 3 BL (excellent) contained more cells than grade 2 BL (good).

Materials and Methods:

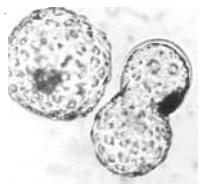
IVM, IVF and IVC: Standard procedures were used for the production of abattoir derived IVP embryos. For IVM, Dulbecco's Modified Eagle's Medium (DMEM Sigma D 5523) was supplemented with 50 ng/mL EGF, 2 U/mL Suigonan Vet® (PMSG-hCG), 5% estrous cow serum (ECS) and 50 µg/mL gentamicin. For IVF, TALP medium containing 6 mg/mL BSA (Fraction V), 0.25 mM pyruvate, PHE (Penicillamine 20 µM, Hypotaurine 10 µM, Epinephrine 1 µM), 30 µg/mL heparin (170 USP/mg) and 50 µg/mL gentamicin was used. IVM and IVF were performed in 5% CO_2 in humidified air (20% O_2) at 38.5°C. After 24 h of IVF (Day 1) the cumulus cells were removed, and the presumptive zygotes randomly divided into four IVC treatment groups in SOFaa + 5% ECS (Holm P et *al.* 1999 Theriogenology **52,** 683-700) with or without a 1:200 (v/v) suspension of bovine oviduct cells (BOEC).

Experimental design: The embryos were cultured in 5% CO_2 in humidified air (20% O_2, 75% N_2) in a CO_2 incubator, or in a Modular Incubator Chamber that was flushed with 5% CO_2 in 5% O_2 and 90% N_2, and placed in the same incubator to ensure identical temperatures (38.5°C). Four different IVC groups were compared: SOFaa + 5% ECS + BOEC in either 5% O_2 or 20% O_2, and SOFaa + 5% ECS in either 5% O_2 or 20% O_2. At Day 8 after IVF the total blastocyst rates were assessed per inseminated oocyte **(BL)**, the blastocysts graded for morphology **(M)**, and stage **(K),** and then a number of the excellent and good graded BL were removed for blastomere count.

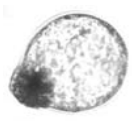
Morphology grading: **3: excellent** (compact and distinct ICM, regular morphology of trophoblast cells, development as expected); **2: good** (smaller or less distinct ICM, a few degenerated trophoblast cells or slight fragmentation; development as expected); **1: poor** (diffuse or no ICM, degenerated trophoblast cells or much fragmentation, developmental arrest). From this information it was possible to calculate the average quality (**M** Morphology).

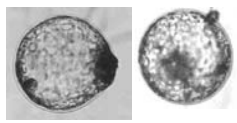
Kinetics: At Day 8 after IVF the embryos were assigned a score according to their developmental stage. **1: BL** (non-expanded BL), **2: XB** (expanded BL), and **3: H** (hatched BL). From this information it was possible to calculate the average developmental stage (**K** kinetics).

Cell count: The zona pellucida and the cytoplasm of the blastomeres were lysed in 0.01 M HCL in 0.1% Tween 20 directly on the glass slide. When all the cytoplasm and debris was dissolved and just before the drop dried out, the isolated nuclei were flushed with 3:1 (v/v) methanol:glacial acetic acid for fixation. The numbers of nuclei were counted using a phase contrast microscope (Viuff D *et al.* 2002 Biol. Reprod. **63**, 1143-1148).

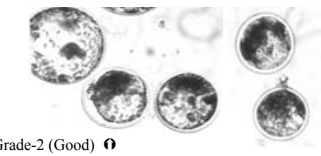


Grade-3 (Excellent)





Grade-2 (Good)



Grade-1 (Poor)

Table 1. Day 8 bovine blastocyst rates, morphology, and kinetics after IVC in SOFaa with 5% estrous cow serum ± coculture with bovine oviduct epithelial cells (BOEC) in 5% CO_2 with high (20%) or low (5%) oxygen tensions at 38.5°C.

IVC Gas	5% CO_2	SOFaa + 5% ECS + BOEC 20% O_2	SOFaa + 5% ECS + BOEC 5% O_2	SOFaa + 5% ECS 20% O_2	SOFaa + 5% ECS 5% O_2
BL rates	BL/ oocyte	33 ± 6.1%[a] 192/588	32 ± 7.8%[a] 180/578	23 ± 9.0%[b] 126/552	30 ± 7.6%[a] 197/655
Morphology	Grade				
Excellent	3.	41 ± 3.7%[a]	22 ± 7.2%[b]	26 ± 6.5%[b]	47 ± 9.9%[a]
Good	2.	41 ± 3.2%	47 ± 14%	50 ± 24%	38 ± 14%
Poor	1.	19 ± 2.5%[a, b]	31 ± 13%[c]	26 ± 20%[b, c]	16 ± 10%[a, d]
Average morphology		2.21 ± 0.05	1.90 ± 0.16	1.98 ± 0.19	2.31 ± 0.14
Kinetics	Grade				
Fast (H)	3.	18%[a] (34)	12%[a] (22)	10%[a] (13)	35%[b] (69)
Medium (XB)	2.	63%[a] (120)	64%[a] (116)	64%[a] (81)	49%[b] (96)
Slow (BL)	1.	20% (38)	23% (42)	25% (32)	16% (32)
Average kinetics		1.96 ± 0.20	1.92 ± 0.11	1.87 ± 0.19	2.16 ± 0.23
B x M x K score		143	117	85	155
Rank		II	III	IV	I

Based on 6 replicates and 2373 inseminated bovine oocytes. Results in rows with different superscripts are significantly different (Fisher's exact test; $P < 0.01$). Results are expressed as Mean ± SD, and as blastocysts per inseminated oocytes. H: hatched; XB: expanded; BL: non-expanded blastocyst; ECS: estrous cow serum.

Table 2. Cell counts (Mean ± SD) for grades 3 (excellent) and 2 (good) bovine IVP blastocysts

Morphology	XB	H
Grade-3 (excellent)	134 ± 50[a] (40)	168 ± 48 (30)
Grade-2 (good)	94 ± 45[b] (62)	143 ± 54 (5)