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Veterinary Medicine

Uptake of $C_{18:0}$ from culture media during *in vitro* culture decreases cryosurvival rates of bovine embryos

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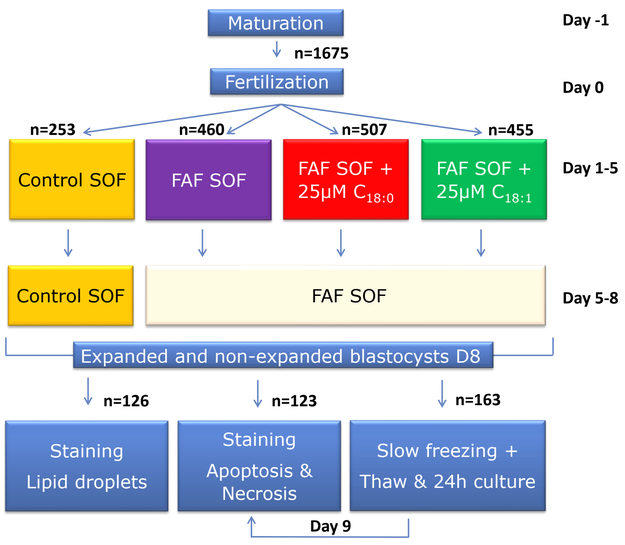
Background

Cryosurvival of *in vitro* produced bovine embryos is reduced compared to *in vivo* produced embryos[1], limiting their usability in the field[2]. Previous work showed that the embryo's lipid composition relates to its quality and cryosurvival[3].

The present study aims to investigate the effects of free fatty acid additions during the oviduct phase of embryo culture on the cryosurvival rate of *in vitro* produced blastocysts.

Methods

Figure 1. Schematic representation of experiment



SOF Synthetic oviduct fluid
FAF Fatty acid free

Bovine cumulus-oocyte-complexes were collected from 2-8 mm follicles of slaughterhouse ovaries and matured and fertilized *in vitro*. Resulting presumptive zygotes were cultured in synthetic oviduct fluid (SOF) until the blastocyst stage. Blastocysts were either stained directly or frozen in an ethylene glycol based medium.

Results

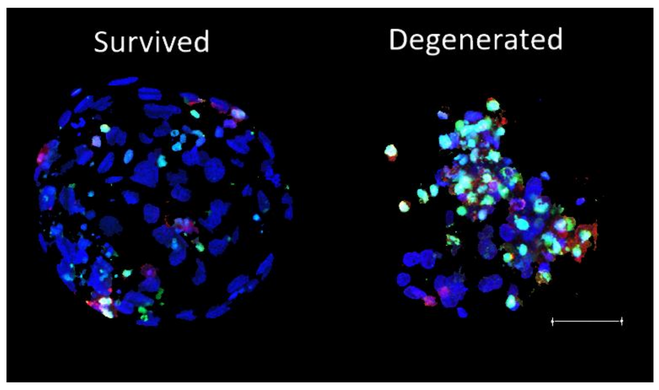
FAF culture delayed and decreased blastocyst rates on culture day 8, compared to any fatty acid supplementation ($p<0.04$, Figure 5). Cryosurvival increased after culture in FAF SOF and $C_{18:1}$ supplementation in comparison to $C_{18:0}$ supplementation ($p=0.01$ & $p<0.01$ resp.) and control ($p=0.15$ & $p<0.02$, resp., Figure 3), approaching cryosurvival rates of donated *in vivo* produced blastocysts (CRV company, The Netherlands; 67%). $C_{18:0}$ exposure also resulted in a higher proportion of damaged blastomeres after cryopreservation, compared to all groups ($p<0,005$, Figure 4). The lipid droplet size increased in blastocysts cultured with $C_{18:1}$ compared to all groups ($p<0.016$, Table 1).

Conclusion

The current data show that free fatty acids have a major impact on the cryosurvival of blastocysts. $C_{18:0}$ addition during *in vitro* embryo culture was detrimental for blastocyst cryosurvival. On the other hand, the total omission of free fatty acids during embryo culture resulted in lower blastocyst rates. Interestingly, $C_{18:1}$ addition resulted in an increase in the lipid droplet size, maintained the blastocyst rate and resulted in a high cryosurvival rate.

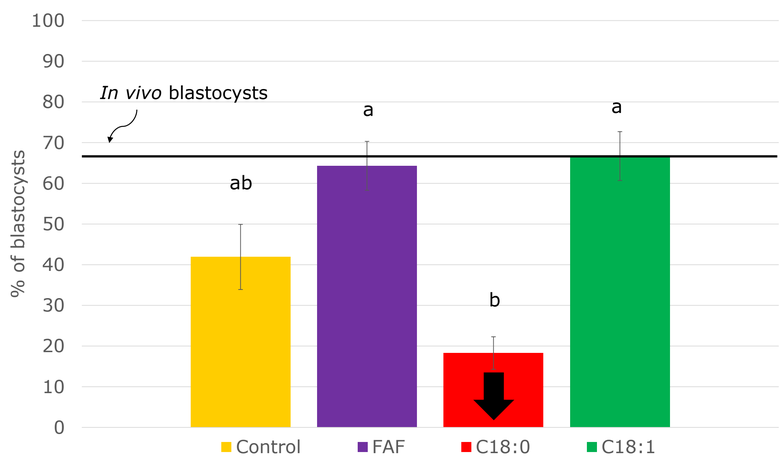
Cryosurvival is lower in blastocyts cultured with $C_{18:0}$

Figure 2 Frozen-thawed blastocysts: apoptosis and necrosis



DNA, necrosis and apoptosis (DNA strand breaks) stained by Hoechst (blue), EthD-1 (red) and TUNEL (green), respectively[4]. From left to right a maximum overlay image of a frozen thawed blastocyst that survived and a degenerated frozen-thawed blastocyst are shown. Scale bar indicates 50 µm.

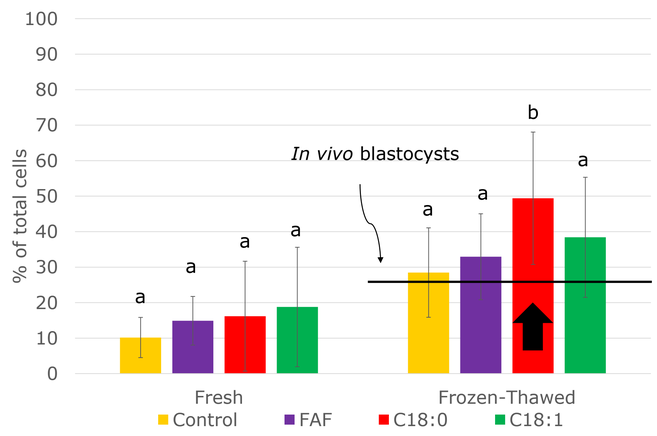
Figure 3 Cryosurvival after a 24-hour revitalization period



Survival is defined as re-inflation of the blastocoel. Data were analysed with a logistic regression model and Tukey test. Different letters on bars indicate significant differences. ($p<0,05$).

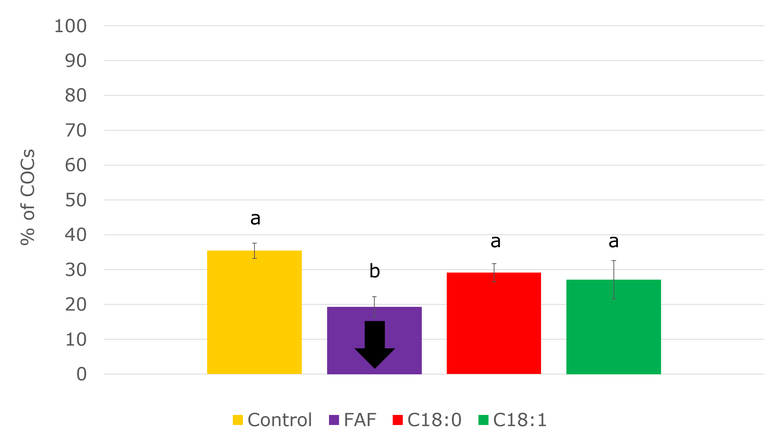
Culture without fatty acids may not be the solution

Figure 4 Percentage of damaged blastomeres per blastocyst



Data analysed using a negative binomial regression and Tukey test. Different letters on bars indicate significant difference ($p<0,05$).

Figure 5 Blastocyst rates on day 8 of culture



Data analysed using grouped logistic regression and Tukey test. Different letters on bars indicate significant difference ($p<0,05$).

Lipid droplet size increased in blastocysts cultured with $C_{18:1}$

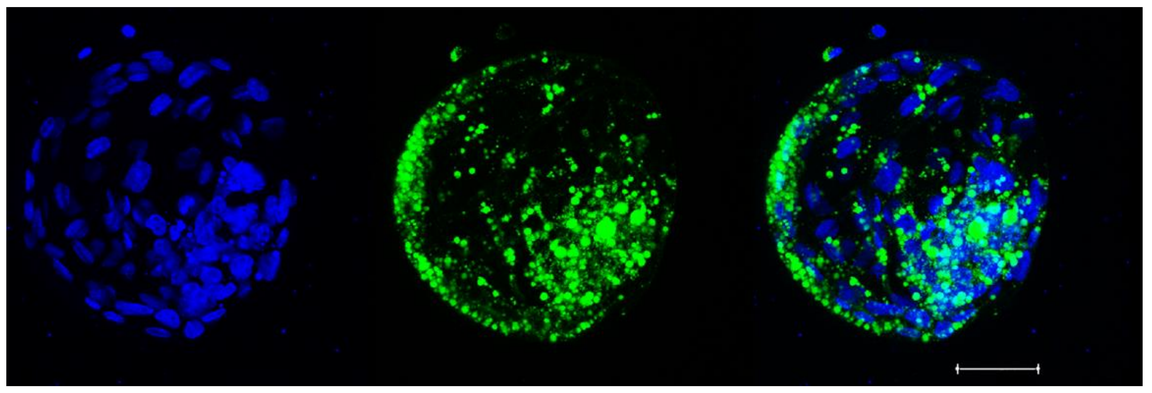


Figure 6
Neutral lipid droplets
Nuclei and neutral lipid droplets, stained by Hoechst 33342 (blue) and LD540 (green); a dye based on the bodipy fluorophore[5], respectively. Maximum overlay images are shown. Scale bar indicates 50 µm.

	n	Number of nuclei per blastocyst ± SD	Number of lipid droplets per blastocyst ± SD	Average lipid droplet size per blastocyst in µm²
Control SOF	24	260.7 ± 75.9[a]	2033.7 ± 545.4[a]	2.66 ± 0.89[a]
FAF SOF	24	207.7 ± 70.8[a]	1655.2 ± 584.9[a]	2.60 ± 1.11[a]
FAF SOF + $C_{18:0}$	42	248.6 ± 81.9[a]	1778.0 ± 615.9[a]	2.45 ± 0.90[a]
FAF SOF + $C_{18:1}$	36	205.8 ± 81.4[a]	1714.7 ± 643.6[a]	3.11 ± 0.75[b]

Table 1
Nuclei- and neutral lipid droplet number and size in day 8 blastocysts
Data analysed using an ANOVA model and Tukey test. Columns with different superscripts differ significantly ($p<0,05$).

References

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