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The R&D letter
22nd January 2016

Deliverable No.1 >
Optimization of conditions for production of OPU-IVP embryos

Under subject 1 "Improvement of fertility" and deliverable S8 "Survival and storage of gametes", the IVF laboratory at the ALLIC phenotyping center recently performed tests for the optimization of the protocol for the production of in-vitro bovine embryos (OPU-IVP). The results were obtained from evaluations of sample ovocytes collected from abattoir ovaries used as part of the research projects financed by or services provided for the company IMV Technologies.

The in-vitro production (IVP) of bovine embryos is a fairly complex reproduction biotechnology requiring control over all stages of production of the embryo. These stages start with the collection of ovocytes in donor females using and Ovum Pick Up (OPU) process and then several stages of maturation, fertilization and in vitro growth of embryos until the blastocyst stage. According to the abundant scientific literature on this subject (see Hoelker et al. 2014, Repro. Fert. Dev. 26 :22-36), bovine embryos produced in vitro present marked morphological, biochemical (lipid composition) and functional (transcriptomics) differences compared to in vivo embryos. These differences are due, in part, to using a sub-optimal system for in vitro culture handling (basic surroundings, serum, mineral oil, etc.).

THREE STUDIES

1. Tests of batches of fetal bovine serum for in vitro maturation (IVM)
2. Test of types of mineral oil for the embryo culture
3. Test of a new type of incubator (provision of service for IMV Technologie)

3. TEST OF A NEW KIND OF INCUBATOR

IVP embryo cultures, until the peri-implantation stage, are grown in vitro in incubators with controlled atmosphere and temperature. These incubators, generally found in laboratories, have a volume of 150 to 200 liters and fluctuations in their internal temperature and atmosphere, particularly from repeated opening of their doors, can compromise the quality of the embryos produced. In recent years, mini-incubators that generally have a volume of under 0.5 liters, which support the stability of environmental parameters for growth even with repeated openings, have been used in many human MAP (medically assisted procreation) laboratories.

The purpose of this experiment, performed on behalf of IMV Technologies, was to compare the potential for developing bovine IVP embryos in two different incubators: the Planer (volume of 0.43 liters; Figure 3) and the Forma by Thermo Scientific (volume of 184 liters). Prior to the embryo culture stage, the ovocytes were matured and fertilized using the same protocol, with the only difference being the conditions they were grown in. The Planer and Forma chambers were opened the same number of times during this test.

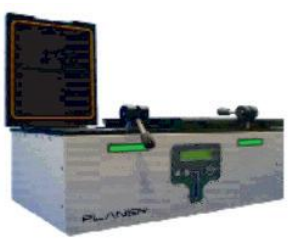

Tableau 3 > ***Results for in vitro development of bovine embryos depending on the type of incubator used for the in vitro culture (4 repetitions).***

Groups	No. of ovocytes	Segmentation rates	% blastocysts D7	% BED7*	% Q1D7**
PLANER	320	90.6±1.6	37.8±3.3	66.4±11.3a	22.5±5.4c
FORMA	320	91,3±1,0	35,9±4,4	53.9±14.6b	15.6±5.3d

a,b: *P* <0.05; c,d: *P* <0.05

*: % of expanded blastocysts at D7; **: % of D7 blastocysts rated 1

Segmentation rate, % D7 blastocysts, %Q1D7 reported in relation to number of ovocytes

% BED7 reported in relation to total D7 blastocysts

Figure 3 > ***Planer Incubator (volume of 0,43 liters)***

This comparative study showed that the frequency of development into blastocyst after 7 or 8 days of culture is not altered by the incubator used. However, the quality of blastocysts was significantly higher for embryos grown in the Planer incubator in relation to those grown in the Forma incubator (Table 3 and Figure 3). The improvement in the quality of the embryos produced in vitro could allow improved gestation rates after transfer of fresh IVP embryos or after freezing and direct transfer. The results obtained from OPU-IVP processes can improve and support development. The in vitro culture of embryos must be performed in a stable environment and the incubator must allow the control of variables such as temperature, atmosphere and humidity. The two incubators studied were different in a number of ways, two of which were particularly interesting for this research. The first is the interior volume.

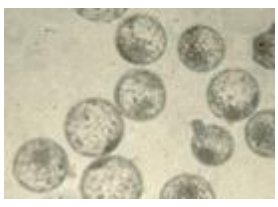

Figure 4 > ***Bovine blastocysts produced in vitro in the Planer BT37 incubator***

The Planer incubator is made up of two independent 0.43 liter chambers, while the Forma incubator has a single 184 liter chamber. The second is the gas supply for the culture chamber. The Planer incubator is fed by a gas mixture (CO_2, N_2 and O_2), while the Forma incubator has feeds of pure gas (CO_2 and N_2) into the air. These two specific features of the Planer incubator allow improved control over variations in temperature and atmosphere inside the chamber after repeated opening of doors and thus minimize the stress experienced by embryos.

The costs for purchasing these two types of incubator are similar (around €10k per incubator). The running costs with gas are an important factor that influences the overall costs for producing embryos. Thanks to the reduced volume of the two Planer incubator chambers, the gas consumption is greatly reduced in relation to the gas consumption of the Forma incubator. As such, the gas costs for the IVP process were €24.00 for the Planer incubator compared to €65.00 for the Forma incubator. This means that for this process the Planer delivers more grade 1 embryos for a reduced cost. Finally, the size of the incubator could be an important factor in choosing an incubator to install in a laboratory for the production of in vitro bovine embryos.

In conclusion, in our experimental conditions, the in-vitro culture of bovine IVP embryos in the Planer incubator improved the quality of embryos produced in relation to the embryo culture in the Forma incubator.

TAKE HOME MESSAGES...

- ensure quality surroundings (SVF and mineral oil)

- control environmental variations during the growth of embryos in vitro

Further info?
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