Bovine Embryo Development Rates are Affected when Oocytes are Matured in Different Vials Containing HEPES/Bicarbonate Buffered Medium

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Introduction

Lab ware for *in vitro* produced embryos is generally made from embryo tested plastic instead of glass. The quality of the plastic is crucial for the outcome, as plastic is often toxic to the gametes. In addition, gas molecules permeate through the plastic at a rate that depends on a variety of factors, such as diffusion coefficient and thickness of the plastic, thus influencing medium pH. Furthermore, medium volume in the vial and the number of cumulus-oocyte-complexes (COCs) are important factors contributing to pH alteration in the medium. Emission of CO_2 from cumulus cell metabolism contributes to a decrease in pH in low gas permeable vials, whereas a low medium volume increases pH in high gas permeable vials. HEPES buffer does maintain pH for a longer period of time, but not indefinitely when bicarbonate is also present.

Objectives

To choose the optimal system for oocyte transportation during maturation (IVM). Blastocyst rates were compared after maturing different numbers of oocytes, 5, 20 and 45, in glass vs plastic vials.

After screening several plastic vials, the least toxic was chosen. Two different maturation medium volumes, 50 % and 95 %, were assessed in the two different vials.

Results

In Experiment 1 the highest blastocyst rates, 40 % and 43 %, were obtained in glass vials with COC numbers between 5 and 20, in 50 % medium volume. The corresponding pH values in the maturation medium were 7.7 and 7.6 respectively, after 21 hours of IVM. The maturation medium pH was 7.3 at the start of IVM.

In Experiment 2 the highest blastocyst rates were also obtained in the glass vials, though not significantly different. In both experiments the lowest blastocyst rates, 28 % and 29 %, were obtained in glass vials with 45 COCs in both medium volume groups. The corresponding pH value in both groups was 7.0. Furthermore, there was a clearly visible pH gradient in the glass vials with 95 % medium volume. See Fig. 2.1.

Experiment 1
50 % medium volume/vial

Figure 1.1

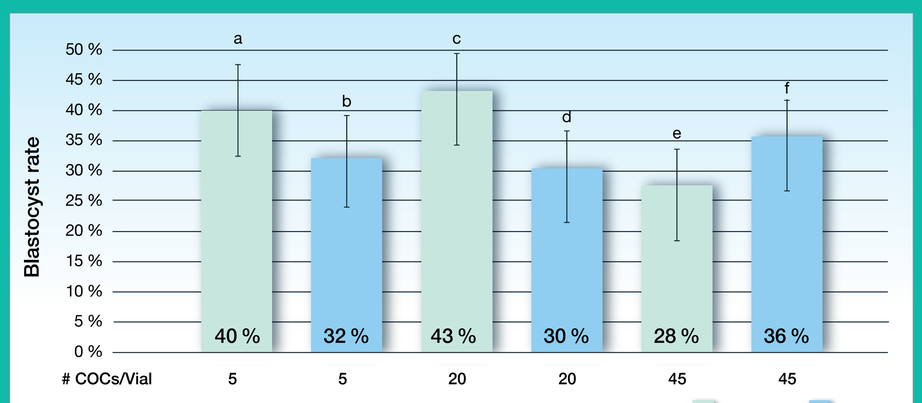

Figure 1.2

# COCs/Vial	COC	Blastocyst %	pH
Glass 5	167	40±7.4	7.7
Plastic 5	171	32±7.0	7.6
Glass 20	245	43±6.2	7.6
Plastic 20	223	30±6.0	7.6
Glass 45	220	28±5.9	7.0
Plastic 45	236	36±6.1	7.5

Table 1.1

Table 1.1, Figure 1.1 and Figure 1.2
Blastocyst rates expressed as percentage of inseminated oocytes (Mean ± SD). Four replicates with a total of 1488 COCs.
Values in a column and within experiment with different superscripts were different
^{a,b}: P ≤ 0.1; ^{c,d}: P ≤ 0.05; ^{e,f}: ≤ P 0.1

Experiment 2
95 % medium volume/vial

Figure 2.1

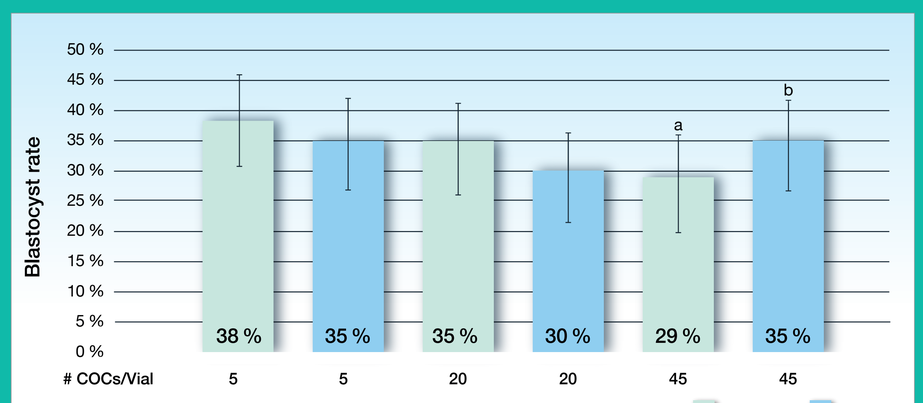

Figure 2.2

# COCs/Vial	COC	Blastocyst %	pH
Glass 5	178	38±7.1	7.3 - 7.5
Plastic 5	196	35±6.6	7.6
Glass 20	212	35±6.4	7.2 - 7.5
Plastic 20	164	30±7.0	7.5
Glass 45	301	29±5.1	7.0 - 7.2
Plastic 45	257	35±5.8	7.4

Table 2.1

Table 2.1, Figure 2.1 and Figure 2.2
Blastocyst rates expressed as percentage of inseminated oocytes (Mean ± SD). Four replicates with a total of 1375 COCs.
Values in a column and within experiment with different superscripts were different
^{a,b}: P ≤ 0.1.

Materials and Methods

pH indicator: A pH indicator guide was created using color-matched photographs for glass (VWR DK, NSCAC 4015-96, 1 ml) and plastic vials (Sigma-Aldrich, CLS430661, 2 ml) containing media at various pH levels with measurements taken using a pH Meter (Fig. 3). The visual pH value estimated from vials was reconfirmed by measuring pH (Fig. 3).

Media: All media used were from IVF Bioscience, UK.

Experiment 1: Slaughterhouse oocytes were randomized into 6 maturation groups: 5, 20 and 45 COCs per vial in glass and plastic, respectively.

- **Matured** (IVM) 21 hours in BO-HEPES IVM, at 38.8 °C without CO_2. Vials contained 50 % medium volume/vial.
- **Fertilized** (IVF) overnight in BO-IVF under a 5.5 % CO_2 in a 38.8 °C humidified atmosphere.
- **Cultured** (IVC) in BO-IVC under a 5.5 % CO_2, 5.5 % O_2, 89% N_2 in a 38.8 °C humidified atmosphere.

Twenty-one hours post IVM the medium pH was assessed from Fig. 3 and presumptive zygotes were vortexed to remove cumulus cells and then cultured in BO-IVC for 7 days (8 days post-IVF) when blastocyst rates were assessed (Table 1.1, 2.1, Fig. 1.2 and 2.2).

Experiment 2: Same protocol as above, but with 95 % maturation medium volume/vial.

Statistical Analysis: Statistical analysis was performed with Chi Square and levels of significance at P ≤ 0.1 and ≤ 0.05.

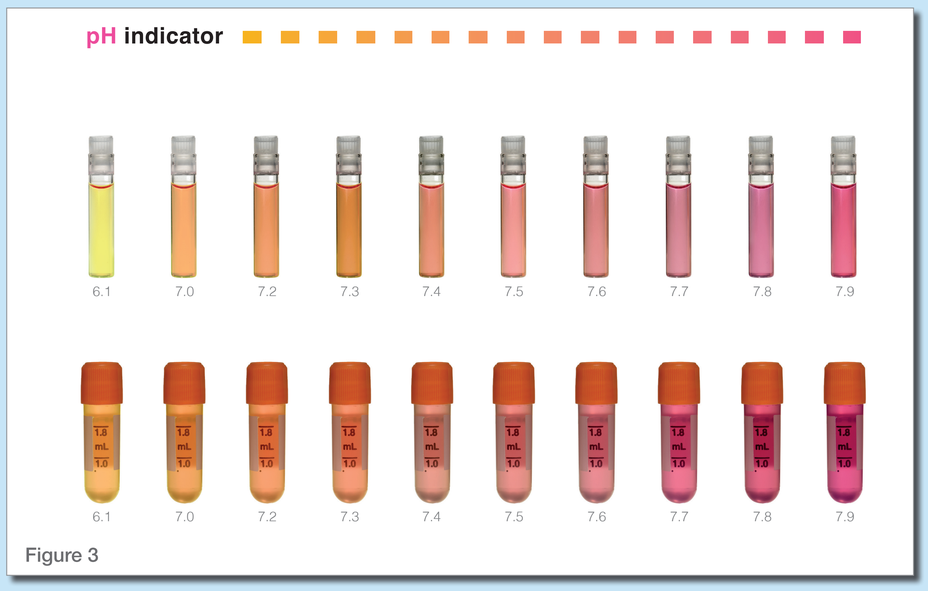

Figure 3

Conclusion

Oocytes can be successfully matured without CO_2 incubation during transport with the selection of the:

- Correct vial
- Correct medium volume
- Correct number of oocytes
- Correct maturation medium

Surprisingly, the acceptable pH range in the maturation medium is rather wide for subsequent good blastocyst rates. The highest rates, 40 %, 43 %, 38 %, and 35 % were obtained in pH 7.7, 7.6, 7.3 and 7.2, respectively.

The toxicity of plastic vials was demonstrated as the glass vial groups showed significantly higher blastocyst rates with more or less the same pH in both vial volume groups with the same number of oocytes, 5 and 20, during maturation.

The gas permeability in the plastic vials was clearly demonstrated as the pH was maintained in all plastic groups at 7.4 - 7.6 independently of number of oocytes and medium volume. In the glass vials with 45 oocytes in both medium volume groups the pH dropped to 7.0, impairing the blastocyst rates. Furthermore, when the medium volume was 95 % a pH gradient occurred (0.2 to 0.3 pH units), with the lowest pH in the medium surrounding the COCs at the bottom of the glass vial.

Final Conclusion:

The optimal blastocyst rates were obtained when oocytes were matured in glass vials in 50 % medium volume/vial and with 5 - 20 oocytes per vial.

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