Comparison of the effect of two different handling media on rabbit zygote developmental ability

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Abstract — Despite the attention paid to culture media, the relevance of the handling medium at egg recovery/transfer is frequently overlooked. In the present work, we compare the effect of two different handling media (PBS and HEPES-buffered Ham F10, both supplemented with 20% (v/v) FCS), upon in vitro and in vivo developmental ability of in vivo fertilised rabbit zygotes. Zygotes recovered in HEPES-buffered medium (permanence 1 h as maximum) and subsequently cultured in vitro developed more efficiently to the compacted morula (100%) and blastocyst stage (92%) than those recovered in PBS (83% and 76%, respectively, \(P < 0.05\)). Zygotes recovered in such media were then further bilaterally transferred to recipient does following a brief in vitro culture period (for 4 hours). At caesarean section (day 28 of pregnancy), significant differences were observed in both the percentage of pregnant uterine horns (PBS: 60% vs. HEPES-buffered Ham F10: 100%) and live birth rates (PBS: 14% vs. HEPES-buffered Ham F10: 34%). Thus when early rabbit zygotes must be handled, even for short incubation periods, the medium is not innocuous.

culture media / buffer system / development / zygote / rabbit

Résumé — Comparaison entre deux milieux de récupération et de manipulation des ovocytes de lapine sur leur capacité ultérieure de développement. Malgré l’attention prête aux milieux de culture, il est fréquent d’oublier l’importance du milieu de manipulation lors de la récupération ou du transfert des œufs. Dans ce travail, nous comparons l’effet exercé par deux milieux de manipulation différents (PBS et HEPES-buffered Ham’s F10, tous deux additionnés de 20 % SVF) sur la capacité de développement in vivo et in vitro des zygotes fécondés in vivo. Les zygotes récupérés dans le milieu tamponné par l’HEPES (1 h de permanence au maximum) et cultivés in vitro présentaient un meilleur développement jusqu’aux stades morula (100 %) et blastocyste (92 %) que les zygotes récupérés dans le PBS (83 % et 76 %, respectivement, \(P < 0.05\)). Dans la seconde expérience, l’effet du milieu

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de manipulation des zygotes sur le développement in vivo a été étudié jusqu’à la naissance. Les zygotes récupérés dans ces deux milieux ont été transférés bilatéralement dans des lapines receveuses après une période de culture in vitro de 4 heures. Après la césarienne à 28 jours de gestation, des différences significatives ont été observées pour les taux de gestation (PBS: 60 % vs. HEPES-buffered Ham’s F10: 100 %) et pour le nombre de jeunes nés vivants (PBS: 14 % vs. HEPES-buffered Ham’s F10: 34 %). En conclusion, quand les zygotes doivent être manipulés, même pour une courte période de temps, le milieu n’est pas inoffensif.

milieu de culture / système tampon / développement / zygote / lapin

1. INTRODUCTION

For embryo production, a number of studies have been focused on in vitro long-term culture [2, 14]. However, handling media and conditions used at egg/embryo recovery or subsequent transfer to recipient does have usually been overlooked.

Brief rabbit embryo handling (corresponding to retrieval from does and/or transfer to recipients) is carried out in balanced usually phosphate buffered simple salt solutions (Hanks, Earle, Dulbecco or Tyrode solution). However, embryo incubation in such simple media for periods longer than 1 hour penalises subsequent in vitro or in vivo developmental ability [1, 22]. For more extended handling periods, the media are usually enriched with bovine serum albumin (BSA, Fraction V), homologous or heterologous sera [8, 11, 22, 25] and even with glucose and/or sodium pyruvate [22]. Despite this, some authors prefer to use more complex, bicarbonate or HEPES-buffered media [3, 8, 9, 21, 26, 28, 32], but to our knowledge, no comparison between simple and complex media has yet been carried out.

In the present work, we compare the effect of two different handling media: Dulbecco phosphate-buffered saline (PBS) and HEPES buffered Ham F-10, both supplemented with 20% (v/v) fetal calf serum, exert upon in vitro and in vivo developmental ability of in vivo-derived rabbit zygotes.

2. MATERIALS AND METHODS

2.1. Source of zygotes

Mature rabbit does from a synthetic line, called H [7], were mated twice with fertile bucks, receiving an intramuscular GnRH injection (20 µg, Fertagyl, Intervet, Spain) at the same time. At 12–13 h after mating, eggs were recovered immediately after euthanasia of donor females by flushing oviducts with either Dulbecco phosphate buffered saline (PBS, D5773, Sigma, Spain) supplemented with 20% (v/v) FCS (hereafter: s-PBS) or with Ham F-10 (N6635, Sigma, Spain) supplemented with 20 mM HEPES (11344, GibcoBRL, LifeTech, Spain) and 20% (v/v) FCS (hereafter: h-Ham). At recovery, the maximum permanence of eggs in these media was 60 minutes.

Collected eggs were incubated in hyaluronidase solution (1 mg.mL⁻¹; H4272. Type IV-S, Sigma, Spain) for 5 minutes to remove the corona cells. Fertilised zygotes were selected for the presence of two clearly defined polar bodies (PB1 + PB2), supernumerary spermatozoa and a healthy general appearance under light microscopy. They were then cultured in bicarbonate-buffered Ham F-10 supplemented with 20% (v/v) FCS (hereafter: s-Ham) in a 7% CO₂ in air and 95% relative humidity and at 39 °C, either for a few hours or for 5 days.

2.2. Experiment 1: in vitro evaluation

In this experiment, we studied the effect of medium used for egg-flushing upon
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At 12–13 hours post-coitum, the recovered zygotes were at an early fertilisation stage, with 77 out of 322 ova showing no signs of fertilisation (fertilisation rate: 76%, data not shown in the tables).

3. RESULTS

At 12–13 hours post-coitum, the recovered zygotes were at an early fertilisation stage, with 77 out of 322 ova showing no signs of fertilisation (fertilisation rate: 76%, data not shown in the tables).

3.1. In vitro developmental ability

The handling media used for oviductal perfusion did not affect the in vitro ability of cultured zygotes (n = 109) to cleave (s-PBS: 97% and h-Ham: 100%; P > 0.05, Tab. I). However, observed significant differences in embryo developmental ability to reach the compacted morula and blastocyst stages indicate that the HEPES-buffered Ham F-10 is a more efficient handling medium (h-Ham: 100% and 92% vs. s-PBS: 83% and 76%, respectively; Tab. I). Such differences in embryo development were also observed at the hatching stage (h-Ham: 78%; s-PBS: 69%); however, they did not reach levels of significance.

3.2. In vivo developmental ability

In this experiment, ten recipient does received a total of one hundred and thirty-six zygotes (average number of transferred embryos per doe: 10.6, ranging from 2 to 10, Tab. II). All ten recipient does became pregnant on the 12th post-ovulatory day and pregnancies progressed to term.

At day 28 of pregnancy, significant differences in the percentage of pregnant...
uterine horns were observed between experimental groups (s-PBS: 60% vs. h-Ham: 100%, \( P < 0.05 \); Tab. II). Moreover, differences in live birth rates per transferred egg were also significant between groups (h-Ham: 34% vs. s-PBS: 14%. \( P < 0.05 \); Tab. II).

4. DISCUSSION

In reproductive biotechnology, amongst several factors, high quality biological material and an efficient egg transfer procedure are required to allow experimentally produced eggs to express their developmental potential, even to term [1, 29].

Zygotes of several species, including rabbits, can be readily cultured to morula or blastocyst stage in vitro [4, 5, 11, 13, 15, 21, 25, 28, this work]. Although in vitro cultured embryos of some species can be successfully transferred to recipient females, successful implantation and development to term in the rabbit depends on mucin-coat

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<tr>
<th>Table I. Effect of two different flushing media on in vitro developmental ability of early rabbit zygotes.</th>
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<td>Recovering medium*</td>
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<tr>
<td>s-PBS 60</td>
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<td>h-Ham 49</td>
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* s-PBS: Phosphate-buffered saline medium supplemented with 20% (v/v) foetal calf serum. h-Ham: HEPES-buffered Ham F-10 supplemented with 20% (v/v) FCS. ¹² Different superscripts within a column differ significantly (\( P < 0.05 \)).

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<tr>
<th>Table II. Effect of two different handling media on in vivo developmental ability of early rabbit zygotes.</th>
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<tr>
<td>Recipient assessment on the 28th post-ovulatory day</td>
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<tr>
<td>s-PBS</td>
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<td>Nb. transferred eggs</td>
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<td>Total</td>
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² Different superscripts between columns differ statistically (\( P < 0.05 \)).
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Egg recovery plus 1 h for transfer) in s-PBS medium are comparable to those obtained by Mills et al. [19] after the immediate transfer of slightly older (24 hpc) rabbit zygotes to synchronised females (12% live pups). Better developmental rates (nearly 34% live born) were obtained in the present experiment, using HEPES-buffered Ham’s F10 (h-Ham) as handling medium. Live pups per transferred egg is comparable to that obtained from more advanced rabbit embryos stages (4- to 8-cell stages), but that are immediately transferred [8, 18, 32]. The in vivo results reported in the present study support observations from in vitro assays, especially considering that embryo culture conditions and media are supported by the recipient doe.

In conclusion, when early rabbit zygotes have to be handled, the medium (in particular phosphate-buffered media) is not innocuous for further development, even for short incubation periods.

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REFERENCES


