SHORT COMMUNICATION

Asynchrony between *in vivo* and *in vitro* rabbit embryos

Asincronía entre embriones *in vivo* e *in vitro* en conejo

Assincronia entre embriões *in vivo* e *in vitro* em coelho

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Abstract

**Background:** The comparative features of embryos that develop under *in vitro* and *in vivo* conditions are particularly important in designing embryo transfer procedures that fulfil embryo-recipient synchronization requirements. **Objective:** To determine the degree of asynchrony in embryo development between cultured and *in vivo* embryos. **Methods:** A total of 55 non-lactating multiparous females were used. Embryos were classified as 16-cells or early morulae at 48 hours post-coitum (hpc). Embryos were cultured during 30 or 32 h and embryo development was

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compared with in vivo embryos of 72 hpc. In vitro and in vivo embryos at 72 hpc were classified as early or compacted morulae. Bayesian statistics was used. Difference between in vivo and in vitro embryos and the actual probability of the difference between the in vivo and in vitro embryo higher than zero (P) was estimated. Results: The percentage of compacted morulae was higher in in vivo embryos than in in vitro embryos with +6 h of asynchrony (73.5 and 32.8%, P=1.00). But the percentage of compacted morulae was similar with +8 h asynchrony. Conclusions: In vitro embryos delay their development by +8 hours compared to in vivo embryos.

**Keywords:** Assisted reproduction; embryo culture; embryonic asynchrony; embryonic development; embryo synchronization; embryo transfer; in vitro embryo production; in vivo embryo production; morulae; rabbits.

**Resumen**

**Antecedentes:** la comparación del desarrollo de embriones producidos in vitro e in vivo es particularmente importante en el diseño de procedimientos de transferencia de embriones en lo que sea necesaria la sincronización entre el embrión y la hembra receptora. **Objetivo:** determinar el grado de asincronía en el desarrollo embrionario entre embriones cultivados e in vivo. **Métodos:** un total de 55 hembras multiparas no lactantes fueron utilizadas. Los embriones se clasificaron en 16 células o mórulas tempranas a las 48 horas después del coito (hpc). Los embriones se cultivaron durante 30 ó 32 horas y el desarrollo embrionario se comparó con embriones de 72 hpc obtenidos in vivo. Los embriones in vitro e in vivo a 72 hpc se clasificaron como móulas tempranas o compactas. Se utilizó estadística bayesiana. La diferencia entre embriones in vivo e in vitro y la probabilidad de que la diferencia sea superior a cero (P) fue estimada. **Resultados:** el porcentaje de mórulas compactadas fue mayor en embriones in vivo que en embriones in vitro con +6 horas de asincronía (73.5 y 32.8%, P=1.00). Pero el porcentaje de mórulas compactadas fue similar con asincronía de +8 horas. **Conclusión:** los embriones cultivados retrasan +8 horas su desarrollo en comparación con los embriones in vivo.

**Palabras clave:** asincronía embrionaria; conejo; cultivo de embriones; desarrollo embrionario; morula; producción de embriones in vitro; producción de embriones in vivo; reproducción asistida; sincronización de embriones; transferencia de embriones.

**Resumo**

**Antecedentes:** a aquisição do desenvolvimento de embriões produzidos in vitro e in vivo é particularmente importante na concepção de procedimentos de transferência de embriões em
que a sincronização entre o embrião e a fêmea receptora é necessária. **Objetivo:** determinar o grau de assincronia no desenvolvimento embrionário entre embriões cultivados e *in vivo.*  
**Métodos:** um total de 55 coelhos multíparos não lactantes foram usados. Os embriões foram classificados em 16 células ou mórulas iniciais 48 horas de gestação (hpc). Os embriões foram cultivados por 30 ou 32 horas e o desenvolvimento embrionário foi comparado com embriões de 72 hpc obtidos *in vivo.* Embriões *in vitro* e *in vivo* a 72 hpc foram classificados como mórulas precoces ou compactadas. Estatísticas bayesianas foram usadas. A diferença entre embriões *in vivo* e *in vitro* e a probabilidade de que a diferença seja maior que zero (P) foi estimada.  
**Resultados:** a porcentagem de mórulas compactadas foi maior em embriões *in vivo* do que em embriões *in vitro* com +6 horas de assincronia (73,5 e 32,8%, P=1,00). Mas a porcentagem de mórulas compactadas foi semelhante com assincronia de +8 horas. **Conclusão:** embriões cultivados atrasam seu desenvolvimento em + 8 horas em comparação com embriões *in vivo.*  
**Palavras-chave:** assincronia embrionária; coelho; cultura de embriões; desenvolvimento embrionário; mórula; produção *in vitro* de embriões; produção de embriões *in vivo*; reprodução assistida; sincronização de embriões; transferência de embrião.
embryonic development of cultured embryos. Therefore, the aim of this study was to determine the degree of asynchrony in embryo development between *in vitro* and *in vivo* embryos at 72 hpc in rabbits.

**Materials and methods**

*Ethical considerations*

All experimental procedures were approved by the Committee of Ethics and Animal Welfare of the Miguel Hernández University (Ref: 2019/VSC/PEA/0017).

*In vivo embryo production and collection*

Non-lactating multiparous females belonging from a maternal rabbit line were used (Argente *et al*., 2019). A total of 41 and 14 does were mating and slaughtered at 48 and 72 h post-coitum (hpc) by intravenous administration of sodium thiopental in a dose of 50 mg/kg of body weight (thiobarbital, B. Braun Medical S.A., Barcelona, Spain). The ovaries, the oviducts and the uterine horns were removed. To recover embryos, the oviduct and the first one-third of the uterine horn were flushed with 5 mL of Dulbecco phosphate buffered saline (DPBS, Sigma, Alcobendas, Madrid, Spain) supplemented with 0.2% (wt/vol) bovine serum albumin (BSA, Cod. A-3111, Sigma) and 0.2 mL of antibiotic (Penivet 1, Divasa Farmavic, Barcelona, Spain) at room temperature. *In vivo* embryos were examined. They were normal if they presented homogenous cellular mass, and spherical zona pellucida and mucin coat. A binocular stereoscopy microscope (Leica Mz 9.5-600x, Wetzlar, Germany) was used. At 48 hpc, normal embryos were classified as 16 cells or early morulae (Figure 1A). At 72 hpc, normal embryos were classified as early morulae or compacted morulae (Figure 1B).

*Cultured embryo*

Normal embryos of 48 hpc from each does were cultured in a one-well 4-well embryo culture dish (NUNC A/S, Thermo Fischer Scientific, Denmark) containing 1 ml culture media (TCM-199 supplemented with 10% fetal bovine serum). Culture was performed at 38.5 ºC in 5% CO₂ in air saturated humidity. *In vitro* developed embryos were examined after 30 or 32 h of culture. So, the asynchrony between *in vivo* and *in vitro* embryos was +6 h or +8 h. Then, embryos were classified as early morulae or compacted morulae. A total of 20 and 21 females were used to culture their embryos with an asynchrony of + 6h and + 8h, respectively.
Statistical analyses

Early morulae and compacted morulae were expressed as a percentage of normal embryos. Differences in asynchrony between in vivo and in vitro embryos were estimated with a model including the effects of asynchrony (in vivo embryos, and in vitro embryos with +6 h and +8 h of asynchrony). Analysis was performed using Bayesian methodology. Bounded uniform priors were used for all effects. Residuals were a priori normally distributed with mean 0 and variance $\sigma^2_e$. The prior for the variance was also bounded uniform. Features of the marginal posterior distributions for all unknowns were estimated using Gibbs sampling. Inferences were derived from the marginal posterior distributions. Median, difference between in vivo and in vitro embryos (D) and the shortest interval with 95% probability of containing the true value (HPD$_{95\%}$) were provide. The HPD$_{95\%}$ showed the precision of the estimation and can be asymmetric around the estimation. The actual probability of the difference between the in vivo and in vitro embryos D higher than zero (P) was estimated. The Rabbit program developed by the Institute for Animal Science and Technology (Valencia, Spain) was used.
Figure 1. A) Scaled *in vivo* early morulae obtained at 48 hpc. B) Scaled compacted morulae from *in vivo* embryos of 48 hpc and cultured for 32 h.

Results

Table 1 shows differences between *in vivo* and *in vitro* embryo development. When the asynchrony was +6 h, percentage of early morulae was lower *in vivo* embryos than *in vitro* embryos (-40.7%, P = 1). So, percentage of compacted morulae was higher. An asynchrony of +6 h was not enough for matching *in vitro* and *in vivo* embryo development. Nevertheless, percentage of early morulae (-7.0%, P = 0.68) and compacted morulae were similar between *in vivo* and *in vitro* embryos with +8 h of asynchrony.

Table 1. Differences between embryo development between *in vivo* and *in vitro* embryos.

<table>
<thead>
<tr>
<th></th>
<th>In vivo</th>
<th>In vitro</th>
<th>D</th>
<th>HPD95%</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>+6 h asynchrony</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(N)</td>
<td>14</td>
<td>20</td>
<td></td>
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<td></td>
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<tr>
<td>Early morulae</td>
<td>32.8</td>
<td>73.5</td>
<td>-40.7</td>
<td>-65.0, -16.3</td>
<td>1.00</td>
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<tr>
<td>Compacted morulae</td>
<td>67.2</td>
<td>26.5</td>
<td>40.7</td>
<td>16.1, 66.2</td>
<td>1.00</td>
</tr>
<tr>
<td>+8 h asynchrony</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(N)</td>
<td>14</td>
<td>21</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early morulae</td>
<td>32.8</td>
<td>39.8</td>
<td>-7.0</td>
<td>-36.0, 18.3</td>
<td>0.68</td>
</tr>
<tr>
<td>Compacted morulae</td>
<td>67.2</td>
<td>60.2</td>
<td>7.0</td>
<td>-20.1, 34.1</td>
<td>0.68</td>
</tr>
</tbody>
</table>

N=number of does; *In vivo*=median of *in vivo* embryos at 72 hpc; *In vitro*=median of *in vitro* embryos; D=median of the difference between the *in vivo* and *in vitro* embryos; HPD95%=highest posterior density region at 95%; P=probability of the difference being >0 when D>0 and probability of the difference being <0 when D<0.
Discussion

The low embryonic development in cultured embryos agrees with other species such as cattle (Lonergan et al., 2016) and pigs (Fowler et al., 2018). Note that embryo culture systems are static, whereas *in vivo* embryos are exposed to a constantly changing environment as it passes along the oviduct to the uterus. Concomitantly, the embryos themselves exhibit a changing physiology and energy metabolism which occur between fertilization and the blastocyst (Gardner, 1998). Thus, embryo manipulation and embryo adaptation to *in vitro* conditions as its requirements change during development lead to a reduction in embryonic development (García, 2018). In rabbits, we have determined that the asynchrony is 8 hours between cultured embryos and *in vivo* ones. This result will allow optimizing the design of experiments in which cultured rabbit embryos are used.

In conclusion, *in vitro* embryos development was lower than *in vivo* embryos. The asynchrony between *in vivo* and *in vitro* embryonic development was 8 h.

Declarations

Funding

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Conflicts of interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

Author contributions


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