Inter-subspecies Somatic Cell Nuclear Transfer between Swamp Buffalo and River Buffalo (Murrah breed)

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Introduction
An inter-subspecies animal is the same species that lives in different areas because of the geographical distribution. They can mate with each other and generate offspring (3). Swamp buffalo and river buffalo are both of genus Bubalus, while belong to different subspecies (5).

Water buffalo (Bubalus bubalis) is broadly classified into river and swamp buffalo subspecies. The swamp buffalo, which is mainly raised for work power, has 48 chromosomes. The river buffalo, which is large-sized and primarily used for milk production, has 50 chromosomes (6). Donor cells of fibroblast origin are easier to reprogram than those of epithelial origin in interspecies SCNT (2).

Inter-subspecies SCNT of transferring river buffalo somatic cells into swamp buffalo oocyte cytoplasm can make full use of swamp buffalo resources to produce river buffalos (5).

There is very little report on this work. The objective of this work was to examine the ability of somatic cell nuclei between swamp buffalo and river buffalo (Murrah breed).

Materials and Methods
Preparation of donor cells: Biopsy of ear skin tissues were taken by using ear notcher from an adult male Murrah and a swamp buffalo at a private farm. Murrah and swamp buffalo ear skin fibroblasts (kept in liquid nitrogen until use) from passages 5-7 were used as donor cells for inter-subspecies into swamp and Murrah buffalo oocytes. Cells were cultured in MEM with 10% FBS and 0.06 g/ml penicillin G and 0.1 g/ml streptomycin in a humidified atmosphere of 5% CO₂, 5% O₂, and 90% N₂ at 38.5 °C. The rest of procedure was previously described by (4).

In vitro maturation of oocytes: Swamp buffalo oocytes were taken from slaughterhouse. Murrah oocytes were taken from a private farm. Oocyte maturation was carried out as previously described (4).

Enucleation: The oocytes were washed 5 times with Hepes-buffered TCM199 plus 20% FBS and enucleated by micromanipulation. Briefly, the oocytes were placed in a small drop of TCM199 plus 10% (v:v) FBS, 12.5 mM Hepes and 5 µg/ml of cytochalasin B. Oocytes with extruded first polar body (metaphase II arrest, MII) were selected for enucleation. The zona pellucida above the first polar body was cut with a glass needle, and a small volume of adjacent cytoplasm was removed, preferably together with the first polar body. Complete enucleation was confirmed by staining with Hoechst 33342 (4).

Nuclear transfer, fusion and activation: A single donor cell (diameter 14-16 µm, with smooth membrane surface) was injected into perivitelline space of enucleated oocytes from 50-µl culture drops of TCM199+12.5 mM Hepes and 10 % FBS (v:v). Four types of couplets were reconstructed as follows:

Murrah – swamp couplets: fibroblast cells from a Murrah buffalo were transferred into the enucleated swamp buffalo oocytes.

Murrah – Murrah couplets: fibroblast cells from a Murrah buffalo were transferred into the enucleated Murrah oocytes.

Swamp – swamp couplets: fibroblast cells from a swamp buffalo were transferred into the enucleated swamp buffalo oocytes.

Swamp – Murrah couplets: fibroblast cells from swamp buffalo were transferred into the enucleated Murrah buffalo oocytes.

For electro-fusion, the oocyte-fibroblast complexes were placed individually between electrodes and were induced to fuse with 2 DC pulses of 30 V for 15 µsec each by electro cell fusion (BTX Electro manipulator Cell 200 San Diego, CA) in Zimmerman fusion medium (7). The electrical pulse also simultaneously induced initial oocyte activation (1). And the nuclear transferred embryos were washed 7 times with TCM199+12.5 mM Hepes + 20% FBS for 45 min at 38.5 °C in a humidified atmosphere of 5% CO₂. Fusion was then confirmed by microscopic examination. All fused embryos were further activated by culturing with 50-µL drops of TCM199+12.5 mM Hepes+10% FBS +5 µM Calcium Ionophore (Sigma A23187) for 5 min. The fused embryo/ reconstructed embryo were washed 5 times and SOFaa + 10% FBS (v:v)+10 µg/mL Cycloheximide (Sigma) + 1.25 µg/mL cytochalasin D (Sigma) at 38.5 °C in a humidified atmosphere of 5% CO₂ for 5 h (4).
In vitro culture of nuclear transfer embryos:

The nuclear transfer embryos were cultured in 50-μl drops of SOFaa + 1% FBS, overlaid with mineral oil, and incubated at 38.5 °C in a humidified atmosphere of 5% CO₂, 5% O₂ and 90% N₂ for 48 h. Cleavage rates were recorded and the eight-cell stage embryos were selected and co-cultured with buffalo oviductal epithelial cells of SOF + 5% CO₂. The medium was replaced every 2 days (4).

Results and Discussion

As shown in Table I, the MII rates and fusion rates were not significantly different among four groups. The cleavage rates till blastocyst rate of reconstructed embryos among the four groups were not different significantly. Our blastocyst rates of swamp cell–swamp oocyte and swamp oocyte–Murrah fibroblast are not different from Yang CY et al (6). Our results showed the developmental competence of swamp–Murrah buffalo NT embryos is not different from that of swamp-swamp embryos that agreed with Yang CY et al (6). Our results showed that inter-subspecies SCNT is possible to produce swamp-Murrah embryos derived from swamp donor cell with Murrah oocytes and Murrah cell with swamp oocytes. This study demonstrated nuclear–cytoplasmic related significantly not affects the early development of reconstructed embryos till blastocyst. Our results suggested that liquid nitrogen storage of buffalo and Murrah ear skin fibroblast cell could support the development of oocytes and subsequently reconstructed embryos. Our results demonstrated that cytoplasm of swamp buffalo could induce the differentiation of frozen cell nuclei derived from Murrah buffalo and support the reconstructed to develop till hatched blastocyst stage and vice versa. However, this study showed that the genetic background of swamp buffalo and Murrah were possibly very similar. In conclusion, inter-subspecies SCNT of transferring swamp buffalo somatic cells into Murrah buffalo oocyte cytoplasm and of transferring Murrah somatic cells into swamp buffalo oocyte can produce reconstructed embryos.

Table 1 Efficiency of inter-subspecies in swamp buffalo and River buffalo

<table>
<thead>
<tr>
<th>Treatments</th>
<th>MII (%)</th>
<th>Enucleated oocytes</th>
<th>No. of fused oocytes (%)</th>
<th>No. of embryos</th>
<th>No. of embryos Cleaved (%)</th>
<th>No. of morula (%)</th>
<th>No. of blastocysts(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>River vs swamp</td>
<td>391</td>
<td>379</td>
<td>314 (82.85)</td>
<td>314</td>
<td>176 (56.10)</td>
<td>80 (25.48)</td>
<td>40 (12.74)</td>
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<td></td>
<td>(54.08)</td>
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<tr>
<td>River vs river</td>
<td>59</td>
<td>56</td>
<td>45 (80.36)</td>
<td>44</td>
<td>28 (63.64)</td>
<td>13 (29.55)</td>
<td>4 (9.10)</td>
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<td></td>
<td>(52.68)</td>
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<tr>
<td>Swamp vs swamp</td>
<td>438</td>
<td>401</td>
<td>338 (84)</td>
<td>338</td>
<td>216 (63.91)</td>
<td>107 (31.66)</td>
<td>56 (16.57)</td>
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<td></td>
<td>(51.47)</td>
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<tr>
<td>Swamp vs river</td>
<td>41</td>
<td>37</td>
<td>36 (97.3)</td>
<td>36</td>
<td>15 (41.67)</td>
<td>9 (25)</td>
<td>3 (8.33)</td>
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<td></td>
<td>(44.57)</td>
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</table>

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References