

RESEARCH ARTICLE

Open Access



Lack of effects of ooplasm transfer on early development of interspecies somatic cell nuclear transfer bison embryos

L. Antonio González-Grajales¹, Laura A. Favetta¹, W. Allan King¹ and Gabriela F. Mastromonaco^{1,2*}

Abstract

Background: Successful development of iSCNT (interspecies somatic cell nuclear transfer) embryos depends on complex interactions between ooplasmic and nuclear components, which can be compromised by genetic divergence. Transfer of ooplasm matching the genetic background of the somatic cell in iSCNT embryos is a valuable tool to study the degree of incompatibilities between nuclear and ooplasmic components. This study investigated the effects of ooplasm transfer (OT) on cattle (*Bos taurus*) and plains bison (*Bison bison bison*) embryos produced by iSCNT and supplemented with or without ooplasm from cattle or plains bison oocytes.

Results: Embryos in all groups were analysed for developmental competence that included cleavage rates, ATP content, and expression of nuclear- and mitochondrial- encoded genes at 8–16 cell stage. Interestingly, no significant differences were observed in embryo development, ATP content, and expression of nuclear respiratory factor 2 (NRF2), mitochondrial transcription factor A (TFAM) and mitochondrial subunit 2 of cytochrome c oxidase (mt-COX2) among groups. Thus, although OT did not result in any detrimental effects on the reconstructed embryos due to invasive manipulation, significant benefits of OT were not observed up to the 8–16 cell stage.

Conclusions: This study showed that a viable technique for OT + SCNT is possible, however, further understanding of the effects of OT on blastocyst development is necessary.

Keywords: ATP, Gene expression, Micromanipulation techniques, Mitochondria

Background

Ooplasm transfer (OT) has revealed fundamental insights in the field of reproductive biology. Transferring ooplasm to a female gamete allowed the discovery of maturation promoting factor in frog oocytes [1], provided evidence of ooplasmic control on mouse embryonic arrest at the 2-cell stage [2], and helped female patients with a history of embryonic failure [3] and idiopathic infertility [4]. However, OT and other invasive techniques, such as spindle transfer, have been discouraged in human reproduction due to safety and ethical concerns [5, 6]. On the other hand, animal models offer an unique opportunity to investigate the effects of

ooplasm-derived components on embryonic development and their interactions with nuclear structures, as in the case of embryos produced by interspecies somatic cell nuclear transfer (iSCNT) [7, 8].

Evidence has shown that incompatibilities between nuclear and mitochondrial genomes in iSCNT embryos have a negative impact on embryo development (reviewed by [9]). It has been proposed that proteins encoded by the nuclear genome are unable to recognize specific sequences of the mitochondrial DNA (mtDNA) due to different taxonomic origin [10]. Furthermore, Zhong et al. [11] suggested that embryonic centrosome dysfunction might be caused by incompatibilities between the ooplasm and the somatic cell. Understanding the interactions between nuclear and ooplasmic compartments is essential to determine the potential causes leading to reduced development or embryonic arrest in iSCNT embryos at early cleavage stages.

* Correspondence: gmastromonaco@torontozoo.ca

¹Department of Biomedical Sciences, University of Guelph, 50 Stone Road E, Guelph, Ontario N1G 2W1, Canada

²Reproductive Physiology, Toronto Zoo, 361A Old Finch Avenue, Toronto, Ontario M1B 5K7, Canada

However, the influence of mitochondrial make-up (heteroplasmy vs xenomitochondrial homoplasmy) on embryo development is complicated with many contradictory findings. iSCNT studies have demonstrated the ability of enucleated oocytes to support first mitotic division with subsequent failure to activate embryonic genes [12, 13]. In wild cattle iSCNT embryos, evidence of increased developmental arrest compared to SCNT controls was observed at the 8–16 cell stage (gaur: [14]; bison: [15]). Therefore, supplementing ooplasm from the same species as the somatic cell into iSCNT embryos could influence embryo development by transferring species-specific proteins, organelles, and other molecules [8, 14, 16]. However, very few reports have implemented OT to investigate its effects on early development of iSCNT embryos.

Sansinena et al. [17] first documented the use of OT in iSCNT embryos and explored its potential applications to overcome the embryonic arrest occurring at early developmental stages observed in a large proportion of iSCNT embryos. Despite the novel approach used in their experiments, one of the main limitations of this technique is the dexterity required to reconstruct and supplement embryos with a small portion of foreign ooplasm. Consequently, implementation of OT with iSCNT might be technically challenging when large numbers of embryos are required. More recently, OT has been carried out in SCNT embryos to determine the effects of the technique on developmental potential and to replenish ooplasm losses as a result of the enucleation procedure [18]. Although no significant differences were found at the blastocyst stage in Gopalakrishna's study, an improvement in fusion rates and early embryo development was observed in the replenished group.

Previous iSCNT experiments conducted in our laboratory used somatic cell donors from two North American bison subspecies (*Bison bison bison* and *Bison bison athabascae*) and domestic cattle oocytes to investigate potential causes responsible for embryonic arrest commonly observed at the 8–16 cell stage [15, 19]. Numerous analyses performed at this stage of development showed alterations in mitochondrial function (metabolism, apoptosis, transcription) predominantly in bison iSCNT embryos [19]. Recently, we investigated whether incorporation of bison ooplasm into bison iSCNT embryos might affect fundamental processes previously studied, such as energy reserves, gene expression, and development rates. To conduct OT + iSCNT experiments, preliminary results revealed that the additional step required to transfer ooplasm limited the use of OT in conjunction with iSCNT due to increased oocyte lysis and elevated exposure time to environmental factors during micromanipulation. Therefore, the aims of this

study were to: 1) establish a 2-step micromanipulation technique to supplement ooplasm derived from cattle oocytes at metaphase II into cattle SCNT embryos, and to 2) determine the effects of cattle and plains bison ooplasm transfer on in vitro embryo development, ATP content, and expression of nuclear and mitochondrial genes in cattle SCNT and plains bison iSCNT embryos.

Methods

Experimental design

Four experimental groups were included in this study: 1) cattle SCNT (C), 2) cattle SCNT supplemented with cattle ooplasm (C + OT), 3) plains bison iSCNT (PB), 4) plains bison iSCNT supplemented with plains bison ooplasm (PB + OT). Domestic cattle (*Bos taurus*) oocytes were used as cytoplasts in all groups while plains bison oocytes (*Bison bison bison*) were used only for ooplasm supplementation into iSCNT embryos. Cleavage divisions at 2, 4, and 8–16 cell stages were determined at 36, 50, and 80 h post oocyte activation (hpa), respectively. Each experiment was repeated on 3 biological replicates. Reconstructed embryos at 80 hpa (day 4) showing normal morphology were randomly assigned to either ATP or PCR analyses. A fifth group consisting of domestic cattle embryos produced by in vitro fertilization (C-IVF) techniques using cattle sperm was included only when carrying out qPCR experiments as an additional control. Cleavage rates were estimated based on the total number of reconstructed embryos placed in culture.

Chemicals

All chemicals were obtained from Sigma-Aldrich Chemical Co. (St Louis, MO, USA) unless otherwise stated.

Animals

No live animals were used for this study. All animal samples, including ovaries and ear biopsies, were obtained post-mortem from Canadian Food Inspection Agency-approved abattoirs.

Culture and preparation of somatic cells

Somatic cell donors consisted of ear skin fibroblasts from cattle and plains bison adult males. Post-mortem punch biopsies were collected from local abattoirs. Fibroblasts were cultured as described previously [14]. Somatic cell donors were at passages 3–5 and confluent for 2–3 days.

Oocyte collections and in vitro maturation (IVM)

Cattle and plains bison ovaries were obtained from local abattoirs. Collection of cumulus-oocytes complexes (COCs) was performed by follicular aspiration into HEPES-buffered Ham's F-10 supplemented with 2 % steer

serum (Cansera International Inc., Rexdale, ON, Canada). Classification of COCs was performed following the inclusion criteria of Leibfried and First [20]. Oocyte in vitro maturation procedures were performed as described previously [19].

In vitro fertilization (IVF) of cattle oocytes

Frozen-thawed *Bos taurus* semen (EastGen, Ontario, Canada) with known in vitro fertility was used for IVF. Semen was prepared by swim-up in HEPES/Sperm TALP for 45 min at 38.5 °C in a humidified atmosphere of 5 % CO₂ in air. COCs were washed three times in HEPES/Sperm TALP at 22 h post IVM, and transferred to 80 µl droplets containing fertilization medium (IVF-TALP, Caisson Laboratories, North Logan, UT, USA) supplemented with 20 µg/ml heparin under silicone oil (Paisley Products, Scarborough, ON, Canada). A final concentration of 1×10^6 motile sperm/ml was used to fertilize 25–30 COCs per droplet. Co-incubation with sperm was carried out for 16 h at 38.5 °C in a humidified atmosphere of 5 % CO₂ in air. Subsequently, groups of 30 presumptive zygotes were placed in 30 µl drops containing modified synthetic oviductal fluid (SOF) medium (Chemicon-Millipore, Billerica, MA, USA) at 38.5 °C in a humidified atmosphere of 5 % CO₂, 5 % O₂, and 90 % N₂ for 4 days as described by Mastromonaco et al. [21].

Embryo reconstruction and ooplasm transfer by micromanipulation techniques

The micromanipulation techniques included three steps: 1) enucleation of recipient oocyte (cytoplasm), 2) aspiration of donor somatic cell and small volume of donor ooplasm (ooplast) within transfer pipette, and 3) simultaneous deposition of somatic cell and ooplasm within the perivitelline space. Cattle and plains bison oocytes were selected based on the presence of an extruded polar body and homogenous ooplasm under light microscopy 18 h post-IVM. Enucleation of all denuded oocytes (cytoplasts and ooplasts) and somatic cell transfer were carried out as described by Mastromonaco et al. [21] with some modifications. Cytoplasts and ooplasts were manipulated in 40-µl microdroplets containing HEPES-sperm TALP medium supplemented with 5 µg mL⁻¹ cytochalasin B under silicone oil on a stage warmer. Following enucleation, 10 cattle cytoplasts and 2 cattle or bison ooplasts were placed in one 40 µl microdroplet, but at opposite poles to segregate the oocyte types. Donor somatic cells were maintained in a separate 40 µl microdroplet placed 0.5 cm apart. Five somatic cells were aspirated into the end of the transfer pipette (15 µm inner diameter) previously coated with 0.01 % PVA/PVP. Immediately prior to somatic cell transfer, one cell was placed at the pipette tip and 10 to

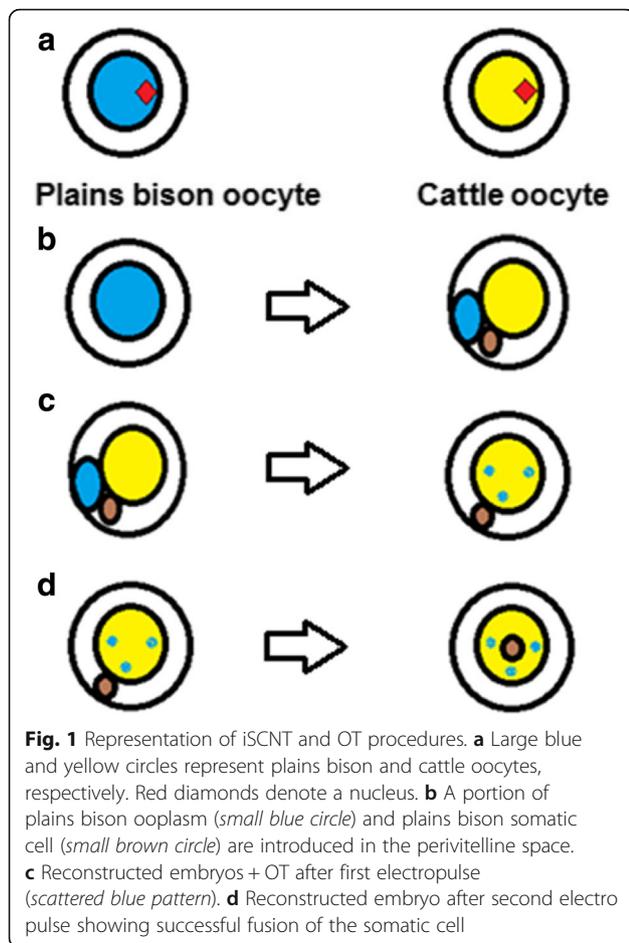
15 % of either cattle or plains bison ooplasm was aspirated. Deposition of ooplasm and somatic cell within the perivitelline space was performed carefully to avoid lysis of the recipient cytoplasm during the procedure. The procedure was repeated until aspiration of more than 70 % of the ooplasm from the ooplast was complete, at which point a new ooplast was selected. Micromanipulations were carried out on an inverted microscope (Leica DM IRB; Leica, Willowdale, ON, Canada). An illustration of a reconstructed embryo including enucleation, transfer of the somatic cell, and ooplasm transfer is shown in Fig. 1.

Fusion, activation and in vitro culture (IVC) of reconstructed embryos

An Electro Square Porator ECM 830 (BTX Harvard Apparatus, Holliston, MA, USA) was used to fuse the somatic cell and a portion of ooplasm in the reconstructed embryo. Fusion of the reconstructed couplets in the case of C and PB groups employed a single DC pulse at 2.3 kV cm⁻¹ for 23 µs. Each reconstructed embryo from C + OT and PB + OT groups received two pulses applied at a 30 min interval. The first DC pulse was set at 1.5 kV/cm for 20 µs to fuse the transferred ooplasm into the reconstructed embryo. A second DC pulse employing higher voltage and longer exposure time (2.1 kV/cm for 32 µs) was used to fuse the somatic cell into the reconstructed embryo once the portion of transferred ooplasm was not longer visible in the perivitelline space. Fusion in all groups was achieved in a 0.5 mm chamber. The fusion solution contained 0.28 M mannitol, 0.01 % bovine serum albumin (BSA), 100 µM MgCl₂ and 50 µM CaCl₂. Micrographs of cattle oocytes before and after fusion of ooplasm are shown in Fig. 2. Oocyte chemical activation protocol was done ~30 min after the second fusion pulse was applied by incubating in HEPES-sperm TALP containing 5 µM ionomycin for 5 min followed by SOF containing 2 M 6-dimethylaminopurine for 4 h at 38.5 °C in 5 % CO₂ with maximum humidity. Lastly, reconstructed embryos were cultured in 30 µl drops of modified SOF covered by silicone oil for 80 h (hpa) using the same conditions mentioned in the previous section.

Adenosine triphosphate (ATP) determination

Accumulated ATP was determined by an adenosine 5'-triphosphate quantification assay (Bioluminescent Somatic Cell Assay Kit; Sigma-Aldrich Canada) in individual embryos from all experimental groups. The protocol was performed as described by González-Grajales et al. [19] while solutions were prepared according to the manufacturer's instructions. Embryos were placed in 10 µl of HEPES-sperm TALP medium, snap frozen and kept at -80 °C until needed. Luminescence reading was



performed using a Fluostar Optima plate reader (BMG Labtech, Ortenberg, Germany). Values for ATP content determined in pmol/embryo were generated from a standard curve containing values ranging from 0 to 5 pmoles. A total of 10 embryos per group were included for this analysis except for C + OT which included 13 samples.

RNA extraction, reverse transcription, and gene expression analysis

Embryos from each experimental group were collected in pools of five at the 8–16-cell stage and washed three times in 0.1 % PBS–PVA, snap-frozen in liquid nitrogen and stored at -80°C until needed. RNA was isolated from pooled embryos using the miRNeasy Micro Kit (QIAGEN, Inc., Burlington, ON, Canada) following the manufacturer's instructions with minor modifications, as previously described [19]. RNA samples were reverse transcribed immediately following extraction using the one-step protocol with qScript cDNA Super Mix (Quanta Biosciences, Gaithersburg, MD, USA) following the manufacturer's instructions. Complementary DNA samples were stored at -20°C until needed. Quantitative real-time polymerase chain reaction (qPCR) was used to measure mRNA expression profiles of selected genes in each experimental group at the 8–16-cell stage. Each transcript analysis was performed on three biological replicates with three technical replicates for each of them, unless otherwise stated. Two of the chosen transcripts (mitochondrial transcription factor A (TFAM) and nuclear respiratory factor 2 (NRF2) belong to the group of genes related to mitochondrial replication, respiration and DNA transcription, all of which are encoded by the nucleus. The third transcript is encoded by the mitochondria (mt-COX2) and encodes subunit 2 of cytochrome c oxidase (complex IV of the electron-transport chain). Glyceraldehyde-3-phosphate dehydrogenase GAPDH was used as a reference gene for all qPCR experiments. All primers were successfully used in *Bos taurus*. The primer sequences and information are listed in Table 1. Specificity of the primers was checked by sequencing and BLAST analysis, as previously described [19]. Quantitative reverse transcription PCR (RT-qPCR) was carried out using the BIO-RAD CFX96 Real-Time PCR System (BIO-RAD Laboratories,

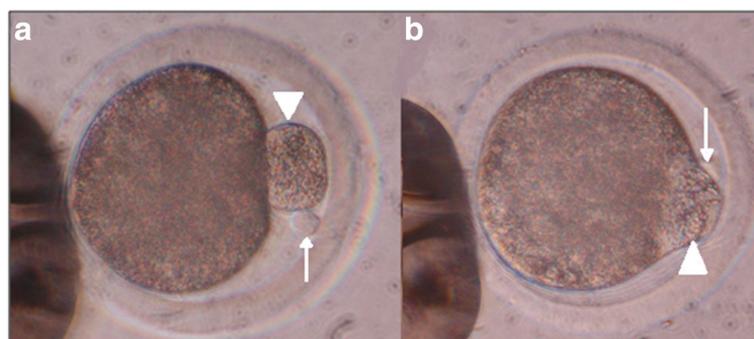


Fig. 2 Phase contrast micrographs of oocytes following ooplasm and somatic cell transfer. **a** cattle oocyte + cattle ooplasm and somatic donor cell nucleus, arrow represents the cattle somatic cell while the arrow head denotes the portion of ooplasm being transferred into the perivitelline space; **b** a different oocyte taken 5 min after first DC pulse. Note fusion process between the cattle oocyte and the portion of cattle ooplasm (*arrow head*) with presence of the somatic cell in the back (*thin arrow*)

Table 1 List of primers used for qPCR experiments

Gene	Genbank accession number	Primer sequence	Product size (bp)	Reference
GAPDH	NM_001034034.2	F: 5'-ttcctggtacgacaatgaatt-3' R: 5'-ggagatggggcaggactc-3'	131	[15]
mt-COX2	3283880	F: 5'-attctgcccgccatc-3' R: 5'-cgtagctcccctggcttt-3'	203	[19]
TFAM	NM_001034016.2	F: 5'-ccgaaaagacctcgctca-3' R: 5'-tctcgtccaacttccatcatt-3'	221	[19]
NRF2	AB162435	F: 5'-tccaaccttggctcatca-3' R: 5'-ttgccgtagctcatctt-3'	174	[14]

Mississauga, ON, Canada) and products were detected with SsoFast™ EvaGreen Supermix (Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacturer's protocols, as previously described [19].

Statistical analysis

Fusion and development rates at 2, 4, 8–16 cell stages were compared using Chi-square in all experimental groups. ATP quantification and gene expression levels were analysed using One-way ANOVA. A probability of <0.05 was considered to be significant. Data were analyzed through the computer program RSTUDIO Version 0.97.551/IC 10.1 (Rstudio, Boston, MA, USA). Data are presented as mean ± SEM.

Results

Fusion rates

The percentage of oocytes with fused ooplasm within 30 min in C + OT and PB + OT groups was 93.0 ± 5.1 and 92.4 ± 2.0 , respectively. After the second pulse (30 min waiting period), rates for fusion of the somatic cell in the former group was 40.76 ± 4.0 ($n = 117$), while for the latter was 53.9 ± 0.7 ($n = 75$). Fusion rates for C and PB were 42.5 ± 3.9 ($n = 91$) and 41.1 ± 3.0 ($n = 111$), respectively. There was a significant difference in the proportion of fused oocytes between PB and PB + OT ($p = 0.01$), however no significant differences were found when C and C + OT groups were compared ($p = 0.6$).

In vitro development of reconstructed embryos

The first three cleavage divisions in all groups (C, C + OT, PB, PB + OT) were monitored to identify differences in development. No significant differences in the proportion of embryos among groups at 2, 4, and 8–16 cell stages were found ($p > 0.05$). Developmental rates for 2, 4, and 8–16 cell stage ranged between 87.5–91.7 ($p = 0.81$), 76.0–83.5 ($p = 0.76$), and 57.6–61.2 % ($p = 0.96$), respectively (Table 2).

ATP quantification

Quantification of ATP was performed in single embryos at the 8–16 cell stage to determine changes in ATP accumulation as a result of ooplasm transfer. ATP content

(pmol/embryo) did not differ among groups ($p > 0.05$). ATP values in C, C + OT, PB, PB + OT were 1.35 ± 0.23 , 1.31 ± 0.34 , 1.27 ± 0.18 , and 1.26 ± 0.31 pmol/embryo, respectively. ATP accumulation from all groups is shown in Fig. 3.

Expression profiles of nuclear and mitochondria-encoded genes

Analysis of expression profiles of gene transcripts was determined by qPCR. Expression of mRNA in embryos supplemented with or without ooplasm and IVF embryos at the 8–16 cell stage was compared to the house-keeping gene GAPDH. The expression profiles for mt-COX2 showed no significant difference among groups ($p = 0.36$). In the case of the other two genes similar expression levels were found for NRF2 ($p = 0.93$), and TFAM ($p = 0.80$) in all groups. Expression profiles for all genes are shown in Fig. 4.

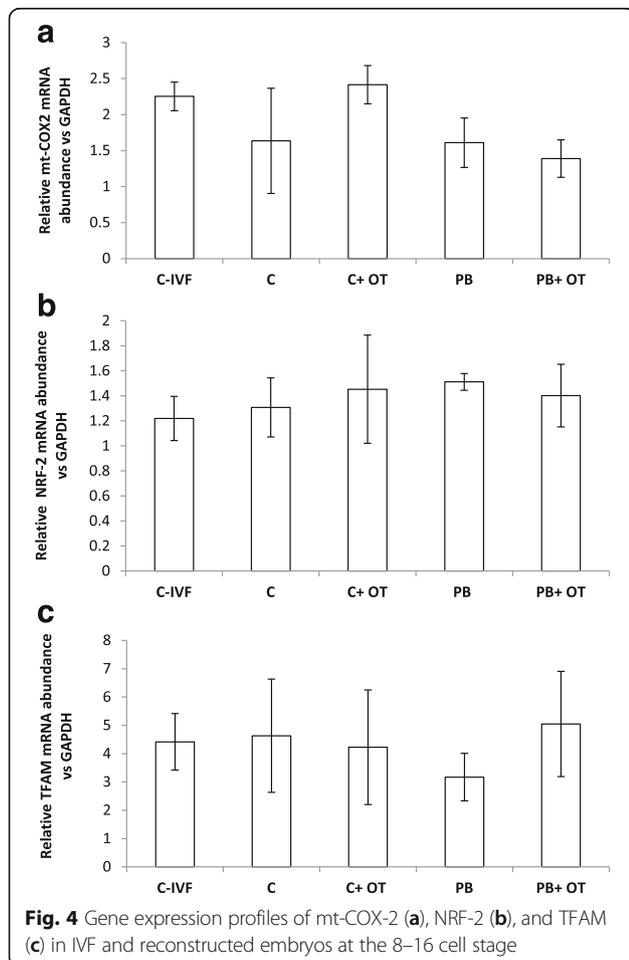
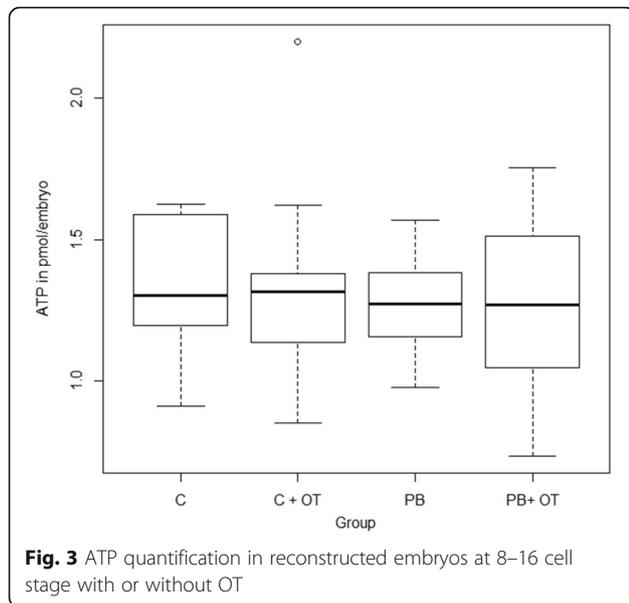
Discussion

It has been hypothesized that ooplasm containing components matching the same taxonomic origin as the somatic cell in iSCNT embryos could influence nuclear-ooplasmic interactions and developmental outcomes [14]. However, limited information is available regarding the effects of OT on early embryo development of iSCNT embryos. Sansinena et al. [17] showed that supplementation of caprine ooplasm in iSCNT embryos (domestic cattle cytoplasm x caprine somatic cell) yielded low fusion and cleavage rates, and poor blastocyst rates (0 %). Their results might have been attributed to technical difficulties since the study lacked a control group (goat ooplasm transferred into goat SCNT embryos). On

Table 2 Percentage of reconstructed embryos developed to the 2, 4, and 8–16 cell stage

Stage of development	C [$n = 85$]	C+ OT [$n = 80$]	PB [$n = 85$]	PB + OT [$n = 50$]	P-value
2-cell stage ^a	91.7 ± 4.0	87.5 ± 2.4	88.2 ± 3.5	88.0 ± 2.4	0.81
4-cell stage ^a	83.5 ± 2.5	80.0 ± 4.9	80.0 ± 6.8	76.0 ± 4.9	0.76
8–16 cell stage ^a	57.6 ± 4.7	61.2 ± 8.1	60.0 ± 7.3	58.0 ± 7.4	0.96

^avalues are reported as mean ± SEM



the other hand, studies applying OT to in vitro produced human and animal embryos have revealed contradictory results since both detrimental and advantageous effects have been attributed to OT [3, 22]. Our investigation showed that a portion of ooplasm transferred into SCNT and iSCNT embryos had no effects on embryo development rates, ATP content, or expression of mitochondrial and nuclear genes in embryos up to the 8–16 cell stage.

Previous studies have successfully transferred and fused ooplasm into oocytes recruited for parthenogenesis and iSCNT experiments [17, 23]. However, combining OT and SCNT requires excessive micromanipulation that may lead to oocyte damage. The technique applied in our experiments reduced the micromanipulation steps resulting in acceptable reconstruction rates evidenced by maintenance of normal ooplasm architecture and high ooplasm fusion rates. Low fusion rates of the somatic cell, both in control and treatment groups resulted in loss of 50 % of reconstructed embryos. Interestingly, more couplets were fused in the PB + OT group than PB group which might be related to increased proximity between the plains bison somatic cell and the oolemma caused by an increase in total ooplasmic volume. Conversely, this was not evident in the C + OT group. Experiments conducted to investigate the causes of low fusion rates obtained in this study did not yield any improvement in fusion rates; therefore, it remains unclear if higher ooplasm volumes resulting from OT enhances fusion in embryos reconstructed by SCNT.

Animal and human OT studies have recommended the transfer of approximately 10 to 15 % of ooplasm volume [3, 23]. A similar approach was used in this study to assess the effects of OT on development, ATP content, and gene expression of iSCNT embryos. Optimal ATP content in oocytes and embryos in different species has been associated with embryo competence since this molecule is involved in several cellular processes during the preimplantation stages (reviewed by [24]). It has been hypothesized that transfer of ooplasm or somatic mitochondrial extract could enhance energy levels in the newly formed embryo resulting in improved development [25, 26]. A study conducted by Van Blerkom et al. [27] showed that fertilized mouse oocytes microinjected with somatic mitochondria accumulated 30 % more ATP 36 h post injection with gradual increases throughout development. The results from our study showed no differences in ATP content among groups 4 days after ooplasm supplementation. This may be explained by the ability of the bovine oocyte to target and eliminate foreign mitochondria at the 8–16 cell stage [28]. Furthermore, the ATP findings obtained in this experiment correlate with embryo development rates at the 2, 4 and 8–16 cell stages ($p > 0.05$).

Improvement in developmental outcomes by OT has been linked to abundance of mRNAs, functional mitochondria, and presence of essential molecules [29]. Mitochondrial transfer into oocytes and embryos has been used to determine the effects of mitochondrial heteroplasmy on subsequent development [27]. Evidence has suggested that addition of mitochondria sharing the same taxonomic origin as the somatic cell might lead to disturbances in iSCNT embryos. This concept was illustrated by Hua et al. [30] who reported impaired development in ovine iSCNT embryos (i.e., ovine somatic cell and domestic cattle cytoplasm) microinjected with mitochondria isolated from ovine granulosa cells. Significantly fewer embryos were able to progress through the 8-cell stage in comparison with iSCNT embryos injected with mitochondria derived from domestic cattle granulosa cells. Their findings demonstrated that heteroplasmy (close to 0.29 %) contributed to poor development in ovine iSCNT embryos injected with ovine somatic mitochondria. Furthermore, the same authors reported down-regulation of pluripotency genes, such as SOX-2 and OCT4, in conjunction with upregulation of TFAM and POLRMT, which are genes involved in mitochondrial replication and transcription [31, 32].

In the last decade, revolutionary disciplines, such as transcriptomics have allowed comprehension of complex processes occurring during early development [33]. Quantification of transcripts in embryos has been used to assess embryo developmental competence *in vitro* and *in vivo* [34, 35]. However, very few OT reports have included PCR studies to understand the interactions taking place between ooplasmic and nuclear components. Thus, we studied expression profiles of genes involved in mitochondrial events such as respiration, transcription and replication (e.g. mt-COX2, TFAM, and NRF2). Our results showed similar gene expression profiles among groups. Moreover cattle IVF embryos were included in this study as an additional control group to investigate expression of these genes at the 8–16 cell stage. Findings from the C-IVF group were in accordance with previous investigations, which documented low expression levels of mitochondrial replication and transcription factors [36, 37]. Interestingly, our study revealed that plains bison ooplasm transferred into bison iSCNT embryos did not alter gene expression of mitochondrial and nuclear encoded genes. Our results suggest that mitochondrial events depending on nuclear and ooplasmic communication are not affected, either positively or negatively, by supplementation of either cattle or plains bison ooplasm at the 8–16 cell stage. In addition, accessibility to plain bison oocytes was limited and restricted to several weeks. Therefore, the number of samples employed in these experiments could also be a limiting factor in establishing significant differences among groups. Further experiments

are required to address these questions and determine if the embryo arrest in bison iSCNT embryos could be ameliorated by implementation of OT.

Conclusions

Application of OT in iSCNT experiments can provide valuable information to understand complex interactions occurring between nuclear and ooplasmic components in reconstructed embryos. However, confounding factors in the ooplasm may mask the beneficial or deleterious effects of supplementing mitochondria contained in a portion of ooplasm. Although our study found no differences at the 8–16 cell stage, further studies are required to understand whether the potential effects associated with OT can be observed at later cleavage stages. Microinjection of mitochondria extract isolated from bison somatic cells, easily available by cell culture, and mature oocytes might provide additional information regarding the role of this organelle in nuclear-ooplasmic incompatibilities.

Abbreviations

ATP: Adenosine triphosphate; BSA: Bovine serum albumin; C: Cattle embryos produced by somatic cell nuclear transfer; C + IVF: Cattle embryos produced by *in vitro* fertilization; C + OT: Cattle embryos produced by somatic cell nuclear transfer supplemented with cattle ooplasm; COCs: Cumulus-oocytes complexes; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase; hpa: Hours post oocyte activation; iSCNT: Interspecies somatic cell nuclear transfer; IVC: *in vitro* culture; IVF: *in vitro* fertilization; IVM: *in vitro* maturation; mt-COX2: mitochondrial subunit 2 of cytochrome c oxidase; mtDNA: mitochondrial DNA; NRF2: Nuclear respiratory factor 2; OT: Ooplasm transfer; PB: Plains bison embryos produced by interspecies somatic cell nuclear transfer; PB + OT: Plains bison embryos produced by interspecies somatic cell nuclear transfer supplemented with bison ooplasm; PBS: Phosphate buffered saline; PCR: Polymerase chain reaction; PVA: Polyvinyl alcohol; PVP: Polyvinylpyrrolidone; SCNT: Somatic cell nuclear transfer; SOF: Synthetic oviductal fluid; TALP: Tyrode's albumin lactate pyruvate; TFAM: Mitochondrial transcription factor A

Acknowledgements

The Authors wish to thank Dr. Monica Antenos, Ed Reyes, Liz St. John, Melissa Filice for their technical support, and all plains bison producers who kindly agreed to participate in this study.

Funding

This study was funded by the Natural Sciences and Engineering Research Council of Canada (GFM), Toronto Zoo Foundation (GFM), Federal Development Agency (WAK), and Canada Research Chairs Program (WAK).

Availability of data and material

Data can be accessed via correspondence with first author.

Authors' contributions

GFM and LAGG developed and designed the experiments. LAGG performed all experiments and statistical analyses and wrote the manuscript. LAF carried out gene expression techniques and GFM performed ATP analysis. WAK and GFM provided funding. All authors were involved in the editing and approval of the manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Experiments were conducted in accordance with the University of Guelph's animal care guidelines for abattoir-derived material.

Received: 5 April 2016 Accepted: 29 September 2016

Published online: 13 October 2016

References

- Masui Y, Markert CL. Cytoplasmic control of nuclear behavior during meiotic maturation of frog oocytes. *J Exp Zool.* 1971;177:129–45.
- Muggleton-Harris A, Whittingham DG, Wilson L. Cytoplasmic control of preimplantation development in vitro in the mouse. *Nature.* 1982;299:460–2.
- Cohen J, Scott R, Schimmel T, Levron J, Willadsen S. Birth of infant after transfer of nucleate donor oocyte cytoplasm into recipient eggs. *Lancet.* 1997;350:186–7.
- Dale B, Wilding M, Botta G, Rasile M, Marino M, Di Matteo L, De Placido G, Izzo A. Pregnancy after cytoplasmic transfer in a couple suffering from idiopathic infertility: Case report. *Hum Reprod.* 2001;16:1469–72.
- Amato P, Tachibana M, Sparman M, Mitalipov S. Three-parent in vitro fertilization: gene replacement for the prevention of inherited mitochondrial diseases. *Fertil Steril.* 2014;101:31.
- Baylis F. The ethics of creating children with three genetic parents. *Reprod Biomed Online.* 2013;26:531–4.
- Amarnath D, Choi I, Moawad AR, Wakayama T, Campbell KHS. Nuclear-cytoplasmic incompatibility and inefficient development of pig-mouse cytoplasmic hybrid embryos. *Reproduction.* 2011;142:295–307.
- Lagutina I, Zakhartchenko V, Fulka H, Colleoni S, Wolf E, Fulka J, Lazzari G, Galli C. Formation of nucleoli in interspecies nuclear transfer embryos derived from bovine, porcine, and rabbit oocytes and nuclear donor cells of various species. *Reproduction.* 2011;141:453–65.
- Mastromonaco GF, González-Grajales LA, Filice M, Comizzoli P. Somatic cells, stem cells, and induced pluripotent stem cells: how do they now contribute to conservation? *Adv Exp Med Biol.* 2014;753:385–427.
- Smith LC, Thundathil J, Filion F. Role of the mitochondrial genome in preimplantation development and assisted reproductive technologies. *Reprod Fertil Dev.* 2005;17:15–22.
- Zhong Z, Spate L, Hao Y, Li R, Lai L, Katayama M, Sun Q-Y, Prather RS, Schatten H. Remodeling of centrosomes in intraspecies and interspecies nuclear transfer porcine embryos. *Cell Cycle.* 2007;6:1509–20.
- Dominko T, Mitalipova M, Haley B, Beyhan Z, Memili E, McKusick B, First NL. Bovine oocyte cytoplasm supports development of embryos produced by nuclear transfer of somatic cell nuclei from various mammalian species. *Biol Reprod.* 1999;60:1496–502.
- Wang K, Beyhan Z, Rodríguez RM, Ross PJ, Lager AE, Kaiser GG, Chen Y, Cibelli JB. Bovine ooplasm partially remodels primate somatic nuclei following somatic cell nuclear transfer. *Cloning Stem Cells.* 2009;11:187–202.
- Mastromonaco GF, Favetta LA, Smith LC, Filion F, King WA. The influence of nuclear content on developmental competence of gaur x cattle hybrid in vitro fertilized and somatic cell nuclear transfer embryos. *Biol Reprod.* 2007;76:514–23.
- Seaby RP, Alexander B, King WA, Mastromonaco GF. In vitro development of bison embryos using interspecies somatic cell nuclear transfer. *Reprod Domest Anim.* 2013;48:881–7.
- Jiang Y, Kelly R, Peters A, Fulka H, Dickinson A, Mitchell DA, St. John JC. Interspecies somatic cell nuclear transfer is dependent on compatible mitochondrial DNA and reprogramming factors. *PLoS One.* 2011;6:e14805.
- Sansinena MJ, Lynn J, Bondioli KR, Denniston RS, Godke RA. Ooplasm transfer and interspecies somatic cell nuclear transfer: heteroplasmy, pattern of mitochondrial migration and effect on embryo development. *Zygote.* 2010;19:147–56.
- Gopalakrishna R, Kumar D, Singh A, Pandey S, Ranjan R, Sarkhel BC. Assessment of developmental potential of caprine cloned embryos with ooplasm replenishment under two culture media. In: *Vitro Cellular & Developmental Biology Animal.* 2014. p. 1–6.
- González-Grajales, L.A., Favetta, L.A., King, W.A., and Mastromonaco, G.F. (2015). Developmental competence of 8–16- cell stage bison embryos produced by interspecies somatic cell nuclear transfer. *Reproduction, fertility and development.* Epub ahead of printing.
- Leibfried L, First NL. Characterization of bovine follicular oocytes and their ability to mature in vitro. *J Anim Sci.* 1979;48:76–86.
- Mastromonaco GF, Semple E, Robert C, Rho GJ, Betts DH, King WA. Different culture media requirements of IVF and nuclear transfer bovine embryos. *Reprod Domest Anim.* 2004;39:462–7.
- Goud AP, Goud PT, Van Oostveldt P, Diamond MP, Dhont M. Dynamic changes in microtubular cytoskeleton of human postmature oocytes revert after ooplasm transfer. *Fertil Steril.* 2004;81:323–31.
- Chiaratti MR, Ferreira CR, Meirelles FV, Méo SC, Perecin F, Smith LC, Ferraz ML, de Sá Filho MF, Gimenes LU, Baruselli PS, Gasparini B, Garcia JM. Xenoplasma transfer between buffalo and bovine enables development of homoplasmic offspring. *Cell Reprogram.* 2010;12:231–6.
- Van Blerkom J. Mitochondria as regulatory forces in oocytes, preimplantation embryos and stem cells. *Reprod Biomed Online.* 2008;16:553–69.
- Malter HE, Cohen J. Ooplasmic transfer: animal models assist human studies. *Reprod Biomed Online.* 2002;5:26–35.
- Yi YC, Chen MJ, Ho JY, Guu HF, Ho ES. Mitochondria transfer can enhance the murine embryo development. *J Assist Reprod Genet.* 2007;24:445–9.
- Van Blerkom J, Sinclair J, Davis P. Mitochondrial transfer between oocytes: potential applications of mitochondrial donation and the issue of heteroplasmy. *Hum Reprod.* 1998;13:2857–68.
- Sutovsky P, Van Leyen K, McCauley T, Day BN, Sutovsky M. Degradation of paternal mitochondria after fertilization: implications for heteroplasmy, assisted reproductive technologies and mtDNA inheritance. *Reprod Biomed Online.* 2004;8:24–33.
- Levy R, Elder K, Ménéz Y. Cytoplasmic transfer in oocytes: biochemical aspects. *Hum Reprod Update.* 2004;10:241–50.
- Hua S, Lu C, Song Y, Li R, Liu X, Quan F, Wang Y, Liu J, Su F, Zhang Y. High levels of mitochondrial heteroplasmy modify the development of ovine-bovine interspecies nuclear transferred embryos. *Reprod Fertil Dev.* 2012;24:501–9.
- Asin-Cayuela J, Gustafsson CM. Mitochondrial transcription and its regulation in mammalian cells. *Trends Biochem Sci.* 2007;32:111–7.
- Ringel R, Sologub M, Morozov YI, Litonin D, Cramer P, Temiakov D. Structure of human mitochondrial RNA polymerase. *Nature.* 2011;478:269–73.
- Uyar A, Torrealday S, Seli E. Cumulus and granulosa cell markers of oocyte and embryo quality. *Fertil Steril.* 2013;99:979–97.
- Cáñeque MJ, Ortega NM, Monteleone MC, Mucci N, Kaiser GG, Brocco M, Mutto A. Expression profile of genes as indicators of developmental competence and quality of in vitro fertilization and somatic cell nuclear transfer bovine embryos. *PLoS One.* 2014;9:e108139.
- Sudano MJ, Caixeta ES, Paschoal DM, Martins A, Machado R, Buratini J, Landim-Alvarenga FDC. Cryotolerance and global gene-expression patterns of *Bos taurus indicus* and *Bos taurus* in vitro- and in vivo-produced blastocysts. *Reprod Fertil Dev.* 2014;26:1129–41.
- Bowles EJ, Lee J-H, Alberio R, Lloyd REI, Stekel D, Campbell KHS, St. John JC. Contrasting effects of in vitro fertilization and nuclear transfer on the expression of mtDNA replication factors. *Genetics.* 2007;176:1511–26.
- May-Panloup P, Vignon X, Chretien M-F, Heyman Y, Tamassia M, Malthiery Y, Reynier P. Increase of mitochondrial DNA content and transcripts in early bovine embryogenesis associated with upregulation of mtTFA and NRF1 transcription factors. *Reprod Biol Endocrinol.* 2005;3:65.

Submit your next manuscript to BioMed Central and we will help you at every step:

- We accept pre-submission inquiries
- Our selector tool helps you to find the most relevant journal
- We provide round the clock customer support
- Convenient online submission
- Thorough peer review
- Inclusion in PubMed and all major indexing services
- Maximum visibility for your research

Submit your manuscript at
www.biomedcentral.com/submit

