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Meta-analysis of trichostatin A treatment effects on mouse somatic cell nuclear transfer

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ABSTRACT

Improving somatic cell nuclear transfer (SCNT) efficiency is challenging, and trichostatin A (TSA) has been implemented to improve this technique, but it does not work for porcine and monkey SCNT. Thus, a meta-analysis was done to understand the relationship between TSA and mouse SCNT. Published articles were collected using PubMed and ScienceDirect from 2000 to 2018. Total 15 studies were included that suggest TSA can improve SCNT mouse blastocyst formation and live birth. Most TSA effects studied were on histone deacetylase (HDACs), hence the impacts of TSA on the cytoplasm, specifically cancer signaling pathways, endoplasmic reticulum, and HDACs localization were investigated. It is likely that TSA benefits mouse SCNT because the nucleus is easy to remove. Using fluorescent labeling to remove nuclei and TSA incorporation, SNCT may be improved for pig and monkey studies.

Key words: Blastocyst, Cloning, Nuclear transfer, Oocyte, Trichostatin A

Improving mouse somatic cell nuclear transfer (SCNT) efficiency is challenging. Trichostatin A (TSA), an acetylation inhibitor, was first used to improve the efficiency of mouse SCNT in 2006 (Kishigami *et al.* 2006), and in 2008 it was used in pigs. We analyzed the effect of TSA on pig SCNT and noted reduced efficiency (Guo *et al.* 2018). In cloned monkeys, TSA improved blastocyst quality but reduced blastocyst formation (Liu *et al.* 2018). To better understand this, we conducted a meta-analysis to explore the relationship between TSA and mouse SCNT.

MATERIALS AND METHODS

Database and data extraction: Literature was collected by two authors independently from peer-reviewed published journals using PubMed and Science Direct. Keywords used to search in PubMed were— (mice OR mouse OR murine OR murines) AND (TSA OR Trichostatin A) AND (SCNT OR clone) AND ("2000/01/01"[PDAT]:"2018/03/01"[PDAT]). ScienceDirect search for pub-date > 1999 and pub-date < 2018 and (mice OR mouse OR murine OR murines) AND (TSA OR trichostatin A) AND SCNT. The articles that were included and their inclusion criteria is given in Table 1. Due to English language limitations, any author conflict regarding summary statistics for key variables reported within the dataset was settled by consultation with a third investigator.

Meta analysis: We assayed the effect of TSA on SCNT efficiency, specifically calculating blastocyst formation from embryonic cleavage and SCNT live births from embryonic transfer. We evaluated effect heterogeneity using Higgins statistic, ap-value, and an I^2 statistic (de la Cruz *et al.* 2017). Briefly, I^2 ranged from 0–100% and heterogeneity of 0–25% was low; 25–50% moderate; and > 50% indicated high heterogeneity (de la Cruz *et al.* 2017). A fixed effects model was used when I^2 was low or moderate. All of the data were calculated using Review Manager, Version 5.3 (Nordic Cochrane Centre, Cochrane Collaboration, Copenhagen). To address publication bias, 3 methods, viz. visual inspection of funnel plots, Egger's test, and Begg's test were used, generated using Stata 12.0 (Stata Corp, College Station, TX) and $P<0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Our search yielded 15 studies from a total of 138 reports (Fig. 1). Table 2 lists descriptive details of every study (Kishigami *et al.* 2006, Kishigami *et al.* 2007, Li *et al.* 2008, Maalouf *et al.* 2009, Tsuji *et al.* 2009, Van Thuan *et al.* 2009, Bui *et al.* 2010, Costa-Borges *et al.* 2010, Dai *et al.* 2010, Ono *et al.* 2010, Hai *et al.* 2011, Kang and Roh 2011, Farifteh *et al.* 2014, Miyamoto *et al.* 2017, Qiu *et al.* 2017). TSA treatment can improve blastocyst rate remarkably [OR 2.01 (95% CI—1.79–2.26)] (Fig. 2). We established that control blastocysts formation was 32.84% (847/2,579), and

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Table 1. Inclusion and exclusion criteria

Inclusion	Exclusion
Species evaluated must include, but are not limited to, mice	Mice were not used
English literature	Non-English literature
TSA treatment of embryos but not limited to embryo treatment	No TSA treatment of embryos
Both donor cell and oocyte came from mice	Xenotransplantation
SCNT blastocyte formation data are available or SCNT birth data are available	Insufficient data

Table 2. Characteristics of studies included in the review

Study	Year	Mouse strain	Treat time (h)*	Medium
Kishigami	2006b	B6D2F1	A 6 + C 4	KSOM
Kishigami	2007a	B6D2F1	A 6 + C 4	KSOM
Li	2008	B6D2F1	O 2 + A 6	KSOM
Tsuji	2009	C57BL/6×DBA	O 2 + A 6	KSOM
Van Thuan	2009	B6D2F1	A 6 + C 4	KSOM
Maalouf	2009	C57/CBA	A 6 + C 4	M16
Biu	2010	B6D2F1	A 6 + C 4	KSOM
Costa-Borges	2010	Hybrid B6CBAF1	O (2–3) + A 6	KSOM
Dai	2010	B6D2F1	O 2 + A 6 + C 2	CZB
Ono	2010	B6D2F1	A 6 + C 3	KSOM
Kang	2011	B6D2F1	A 6 + C 3	KSOM
Hai	2011	B6D2F1 and ICR	A 6 + C 4	MEM
Farifteh	2014	B6D2F1	O 2 + A 6	KSOM
Miyamoto	2017	B6D2F1 and ICR	A 6 + C 2	KSOM
Qiu	2017	Kunming	A 6 + C 4	KSOM

*Treatment time for TSA within different media. O, oocyte culture medium; A, activation medium; and C, embryo culture medium.

TSA improved this (50.64% or 1,312/2,591). Moreover, TSA increased the number of births (Fig. 3) in control and TSA groups by 0.56% (14/2500) and 3.59% (61/1,697), respectively. We did not find heterogeneity. Funnel plot data did not indicate publication bias (Fig. 4), Egger's test P = 0.261, Begg's test Pr> |z| = 0.213. TSA can improve mouse SCNT blastocyst formation and increase live births.

When histone modification changes in SCNT embryos were observed, studies of histone modification of normally fertilized embryos were undertaken. Histone modification includes methylation, acetylation, phosphorylation, ubiquitination, deacetylation, or ADP ribosylation (Shanmugam *et al.* 2018). Two enzymes are involved in the regulation of histone acetylation namely histone acetyltransferase (HATs) and histone deacetylase (HDACs). If cellular acetylation and deacetylation are unbalanced, cell

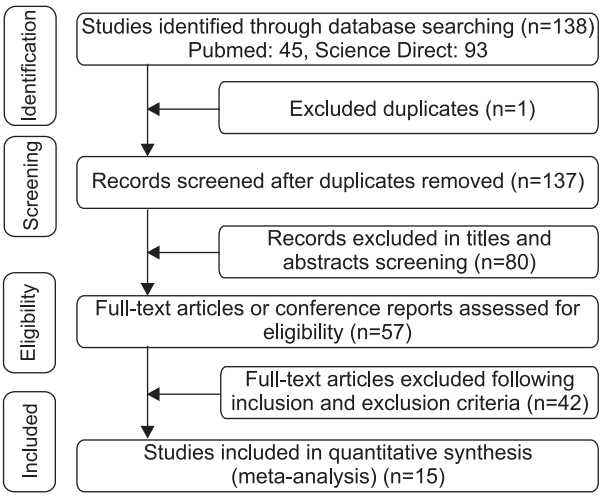

Fig. 1. Summary of study selection.

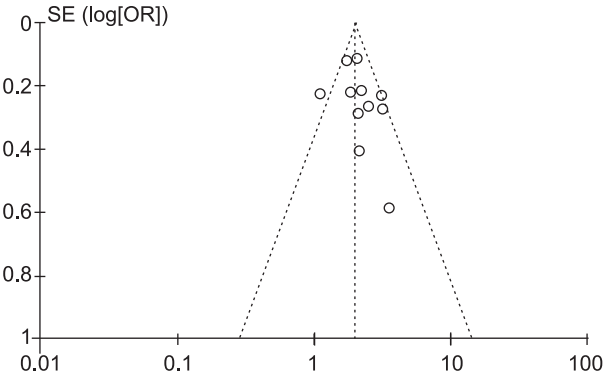

Fig. 4. Funnel plots of TSA-treated mice and mice SCNT blastocyst formation.

proliferation and differentiation are abnormal, and gene expression changes.

Use of TSA on oocytes (Li *et al.* 2008, Dai *et al.* 2010) is controversial as TSA can change HDACs in nuclei (Li *et al.* 2011). When oocyte nuclei are removed, there appears to be no overall effect (Rao and Rao 2013). Therefore, we assessed how TSA affects the cytoplasm, specifically cancer signaling pathways, the ER and HDAC localization. TSA may modify p21 protein expression and prevent the formation of cell cycle two polymer and cyclin-dependent kinase, which can block the cell cycle and induce cell differentiation, contributing to cancer. TSA is also involved in glioblastoma and human nasopharyngeal carcinoma cell p53 pathways. The PI3K/Akt signalling pathway is also linked to TSA. Thus, embryos may be affected in a manner similar to tumor cells.

Studies suggest that TSA affects ER function (Li *et al.* 2017). SER exits in 2 forms in oocytes, viz. vacuoles and small tubular aggregates, and SER is a calcium storage reservoir (Sfontouris *et al.* 2018). Smooth ER aggregate (SERa) is very common in oocytes and calcium ion fluctuation can cause ER stress (ERS). SCNT technology must activate the genome after nuclear transplantation, and

Study or Subgroup	TSA Events	TSA Total	Control Events	Control Total	Weight	Odds Ratio M H, Fixed, 95% CI	Year	Odds Ratio M H, Fixed, 95% CI
Kishigami						Not estimated	2006b	
Kishigami						Not estimated	2007a	
Tsuji	376	730	175	462	226%	1.74 [1.37, 2.21]	2008	
Li	95	154	96	226	7.6%	2.18 [1.43, 3.31]	2008	
Van Thuan	80	129	74	214	5.4%	3.09 [1.96, 4.86]	2009	
Maalouf	44	153	71	266	9.5%	1.11 [0.71, 1.73]	2009	
Dai	49	90	60	185	4.6%	2.49 [1.49, 4.17]	2010	
Costa-Borges	82	177	58	182	7.9%	1.85 [1.20, 2.84]	2010	
Biu	68	104	53	143	4.0%	3.21 [1.89, 5.44]	2010	
Ono						Not estimated	2010	
Hai	445	776	207	520	27.1%	2.03 [1.62, 2.55]	2011	
Kang	21	80	4	44	1.0%	3.56 [1.14, 11.15]	2011	
Farifteh	12	44	24	162	1.9%	2.16 [0.98, 4.76]	2014	
Qiu	40	154	25	175	4.4%	2.11 [1.21, 3.67]	2017	
Miyamoto						Not estimated	2017	
Total (95% CI)		2591		2579	100.0%	2.01 [1.79, 2.26]		
Total events	1312		847					

Heterogeneity: $\chi^2 = 16.77$, df = 10 (P = 0.08); $I^2 = 40\%$
Test for overall effect: Z = 11.61 (P<0.00001)

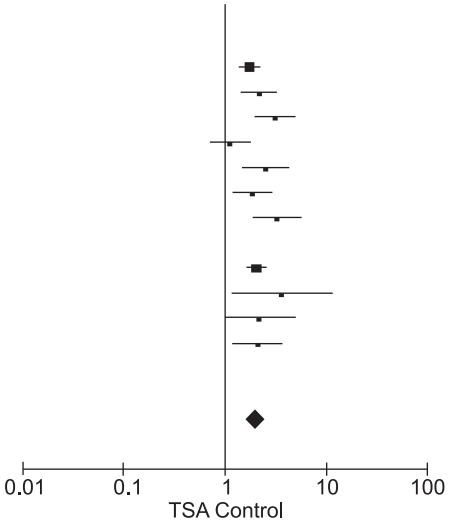

Fig. 2. Forest plot of TSA-treated mice and mice SCNT blastocyst formation. CI, 95% confidence interval.

Study or Subgroup	TSA Events	TSA Total	Control Events	Control Total	Weight	Odds Ratio M H, Fixed, 95% CI	Year	Odds Ratio M H, Fixed, 95% CI
Kishigami	18	287	1	297	8.5%	19.81 [2.63, 149.37]	2006b	
Kishigami	5	120	20	115	4.5%	11.00 [0.60, 201.22]	2007a	
Li						Not estimated	2008	
Tsuji	5	236	0	82	6.7%	3.92 [0.21, 71.67]	2008	
Maalouf	6	319	1	568	6.5%	10.87 [1.30, 90.69]	2009	
Van Thuan	6	80	1	74	8.9%	5.92 [0.70, 50.39]	2009	
Ono	5	120	6	229	36.5%	1.62 [0.48, 5.41]	2010	
Costa-Borges	3	190	0	328	3.3%	12.26 [0.63, 238.72]	2010	
Dai	3	117	4	486	14.0%	3.17 [0.70, 14.37]	2010	
Biu	5	104	1	143	7.4%	7.17 [0.83, 62.33]	2010	
Kang						Not estimated	2011	
Hai						Not estimated	2011	
Farifteh						Not estimated	2014	
Miyamoto	5	124	0	178	3.6%	16.43 [0.90, 299.91]	2017	
Qiu						Not estimated	2017	
Total (95% CI)		1697		2500	100.0%	6.25 [0.90, 299.91]		
Total events	61		2500					

Heterogeneity: $\chi^2 = 7.99$, df = 9 (P=0.54); $I^2 = 0\%$
Test for overall effect: Z = 6.04 (P<0.00001)

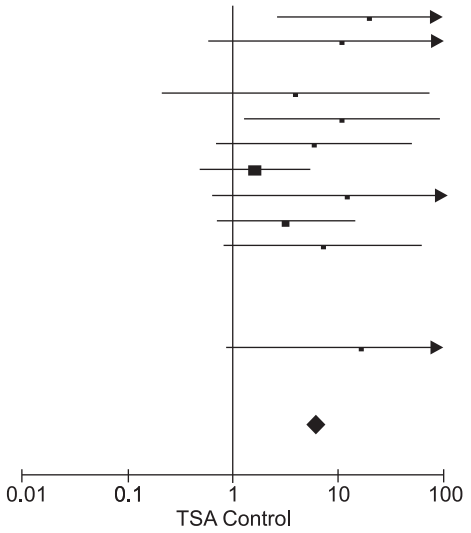

Fig. 3. Forest plot of TSA-treated mice and mice SCNT birth. CI, 95% confidence interval.

this causes calcium fluctuations.

Why TSA is effective for mouse SNCT but not for porcine or monkey SCNT is of interest (Guo *et al.* 2018, Liu *et al.* 2018). Likely, the removal of mouse oocyte nuclei are easier than for other species; they are easily visualized using differential interference contrast (DIC) microscopy. For pig and monkey studies, the nuclear position is difficult to estimate. Thus, nuclear removal significantly reduces the cytoplasm. With the greater cytoplasmic loss, cloning efficiency is reduced. Thus, we assessed porcine hand clones and focused on nuclear/oocyte factors, the ER, and spindle wire. Other studies reported that proximity to the nucleus, the size of the spindle wire, and nuclear factor concentration (Ryu *et al.* 2017). SERa are more distributed near the nucleus (Itoi *et al.* 2016), and excessive cytoplasmic removal near the nucleus will reduce ER content.

TSA can increase mouse SCNT efficiency if fluorescent labeling is used to remove nuclei. This finding may be applied to porcine and monkey SCNT.

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