



Conservation of gaur (*Bos gaurus*) by inter-species somatic cell nuclear transfer

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Cloning procedures

Cytoplasm



Nuclear transfer



Donor cell



Embryo transfer



Pregnancy rate after transferred cloned embryos derived from ear fibroblasts of Brahman bull



No. recipient	No. embryos/ recipient	No. recipient pregnant at						Calving
		60 d	180 d	200 d	220 d	240 d	260 d	
39	62/39 (1.6)	14 (36%)	14 (36%)	13 (33%)	12 (31%)	11 (28%)	10 (26%)	10 (26%)



Toomtams 999/1



Toomtams 2



Toomtams 3



Toomtams 4



Toomtams 5



Toomtams 6



Toomtams 7



Toomtams 8





11-12 months old DOB: October-December 2003

>100 calves born from AI using frozen semen of these bulls



Pregnancy and calving after transferred cloned embryos derived from ear fibroblasts of beef and dairy cattle

Donor cells	No. recipients	No. emb./ recipient	No. pregnant at days				Aborted (# fetuses)	Calving (# calves)
			60	150	180	210		
HF (346)	175	298/175 (1.7)	35 (20%)	29 (17%)	24 (14%)	17 (10%)	9 (9)	8 (dead 5, alive 3)
Brahman (SK 180)	59	94/59 (1.6)	18 (37%)	15 (31%)	15 (31%)	12 (20%)	5 (7)	7 (dead 2, alive 5)

7 years



4 months



Paripati et al., 2004. KU Conference



Red Brahman female: 4 months



Five live birth calves; still survive



DOB: 22 May 2007





HF female: 7 years



Three live birth calves; still survive



White Lamphun cattle

(Native cattle in Northern part of Thailand)



Not more than 2,000 heads



White Lamphun cattle SCNT (Not treat TSA)

Experiment	Fused (%)	Cleaved (%)	8C (%)	Mor.-D6 (%)	Blast.-D8 (%)
Parthenogenetic activation	-	66/80 (83)	45/80 (56)	28/56 (50)	16/56 (29)
SCNT	83/120 (69)	79/81 (98)	66/81 (81)	36/79 (46)	25/79 (32)

Result of ET White Lamphun cattle SCNT embryos (Not treat TSA)

No. recipients	No. embryos ET	Pregnant		Calving
		D-60	D-90	
9	14	4 (44%)	2 (22%)	1 (11%)

Parapai, R. et al., 2006. BIODIVA, Hanoi



Cloned White Lamphun cattle calf



DOB: 21 March 2007



Types of SCNT

1. Intra-species SCNT

Same species : Same chromosome numbers
Cattle-Cattle , Sheep-Sheep , Goat-Goat

2. Inter-species SCNT, Inter-generic SCNT

Different species : Different chromosome numbers
Cattle-Gaur , Cattle-Kouprey, Domestic cat-Wild cat



Inter-species SCNT

Mouflon cell – Sheep oocyte



Cloned female mouflon with the sheep foster mother. Two blastocysts were surgically transferred into the sheep recipient seven days after natural estrus. Only one blastocyst implanted, and the mouflon shown in the photo was spontaneously delivered after 155 days of pregnancy.

Lol et al. Nature Biotechnology (2001), 19: 962 – 964.



Intra-species SCNT

Horse-Horse



“Prometea”
Birth 28 May 2003

Inter-species SCNT

Mule cell – Horse oocyte



2003



Inter-species SCNT

Gaur cell – Bovine oocyte



January 8, 2001 Noah, a baby bull gaur, became the first clone of an endangered animal.

no more report of live birth of iSCNT gaur



Inter-species SCNT

Banteng cell –bovine oocyte



April 2, 2003



iSCNT in gaur at Embryo Technology and Stem Cell Research Center

Gaur cell - Bovine oocyte



Sab



Biopsy gun



Collected tissue



Tong



7-days old embryos



Stained embryonic cells



Leena



Pregnant 35 days

ET to cattle



Developmental rates of cloned female and male gaur embryos

Donor cell	Fused (%)	No. oocytes	Cleaved (%)	Embryos development			
				8-cell (%)	Morula d-5 (%)	Blast. d-6 (%)	Blast. d-7 (%)
Female	130/150 (86.7)	124	102/124 (82.3)	88/102 (86.3)	47/102 (46.1)	33/102 (32.4)	33/102 (32.4) ^a
Male	159/177 (84.2)	141	135/141 (95.7)	119/135 (88.1)	67/135 (49.6)	39/135 (28.9)	55/135 (40.7) ^b

Sang-Ngam, C. et al. 2005 2nd ARS Thailand.

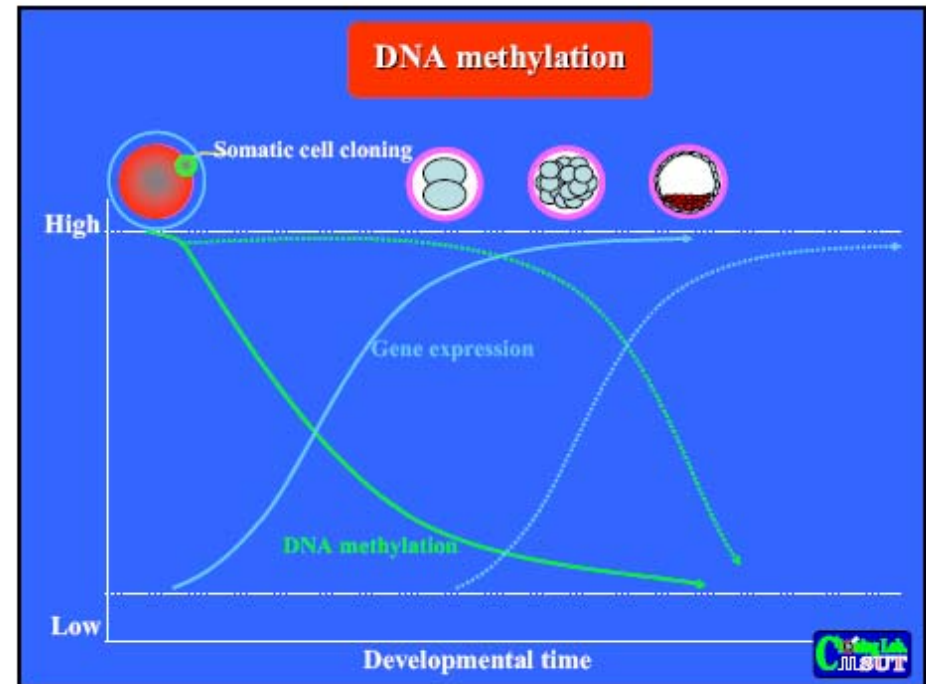
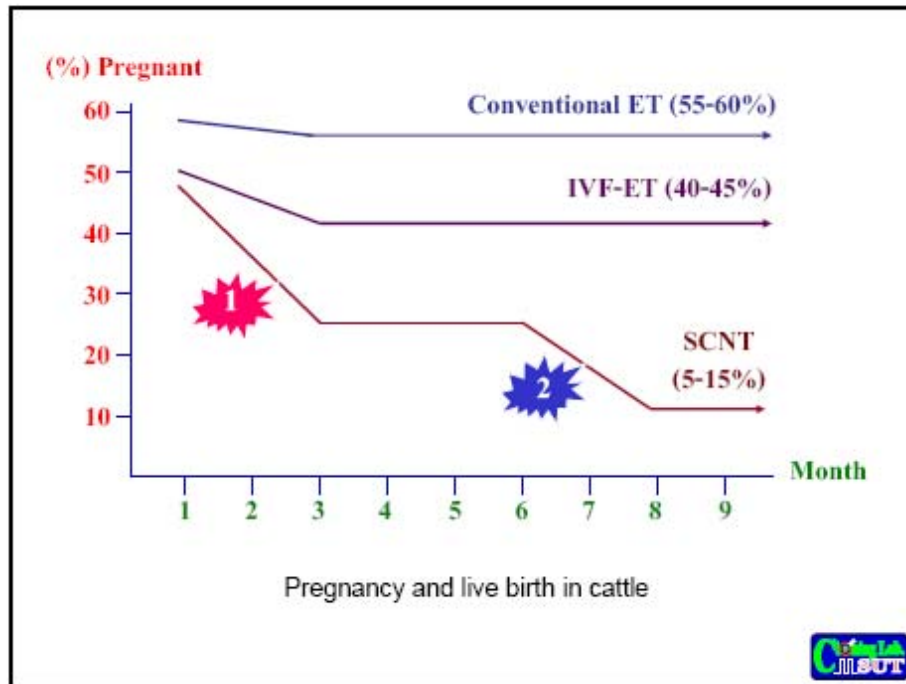


Result of ET iSCNT gaur embryos (non-treat TSA)

Donor cells	Type of embryos	No. embryos	No. recipients	No. pregnant	Final results
Tong (Male)	Fresh	41	20	6 (30%)	4 aborted 2-3 months 1 aborted at 4 months 1 aborted 5 months
Leena (Female)	Vitrified	15	5	1 (20%)	Mummified at 5 months

Pampal, R. et al., 2006. BIODIVA, Hanoi





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Cloning animals by somatic cell nuclear transfer – biological factors

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Trichostatin A (TSA) increased histone acetylation and decreased DNA methylation

Colin COLBUT

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Significant improvement of mouse cloning technique by treatment with trichostatin A after somatic nuclear transfer

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Fusion → 50 nM TSA, 10 h

Colin COLBUT

Effect of TSA on the developmental potential of interspecies cloned gaur embryo derived from male and female skin fibroblasts

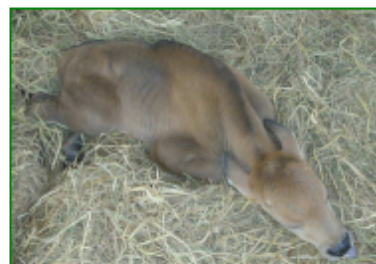
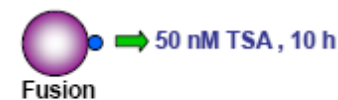
Treatment	Fused (%)	Cultured	Number (%) embryo development (%)			
			Cleaved	S-C	Morula	Blastocyst
Male 0 nM TSA	147/170 (86.5)	146	138/146 (94.5)	119/146 (81.5)	71/138 (51.4)	48/138 (34.8)
Male 50 nM TSA	161/188 (85.6)	157	142/157 (90.4)	114/157 (72.6)	64/142 (45.1)	50/142 (35.2)
Female 0 nM TSA	157/187 (84.0)	154	151/154 (98.1)	123/154 (79.9)	69/151 (45.7)	56/151 (37.1)
Female 50 nM TSA	152/172 (88.4)	151	145/151 (96.0)	120/151 (79.5)	71 (49.0)	57 (39.3)

Siratana et al. IETS 2009



Pregnancy after transfer iSCNT gaur embryos derived from male and female fibroblasts treated with TSA

Sex of donor cells	TSA	No. recipients	Pregnant at days								Calving	
			40	60	90	120	150	180	210	240		270
Male	-	10	1	1	1	1	1	1	1	1	1	1
	+	18	2	2	1	0	0	0	0	0	0	0
Female	-	10	1	1	0	0	0	0	0	0	0	0
	+	20	6	6	5	3	3	3	3	on going		



DOB: 4 March 2008



VTR cloned gaur birth

If we can get live birth of iSCNT gaur.

**Kouprey is one of our ultimate goal
to discover and repopulate**



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