Bovine embryo production and selection system by ovum pick-up and *in vitro* fertilization

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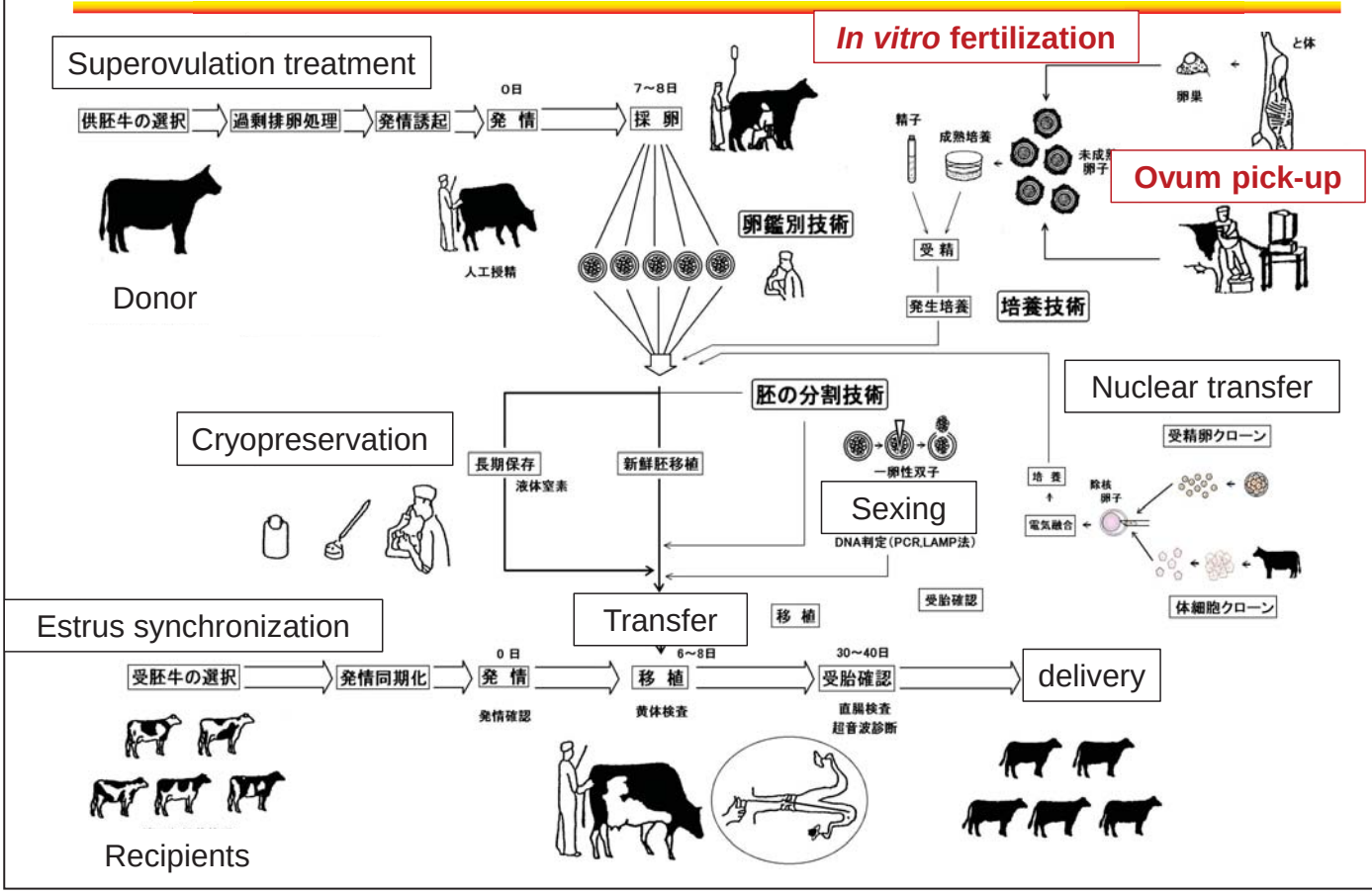
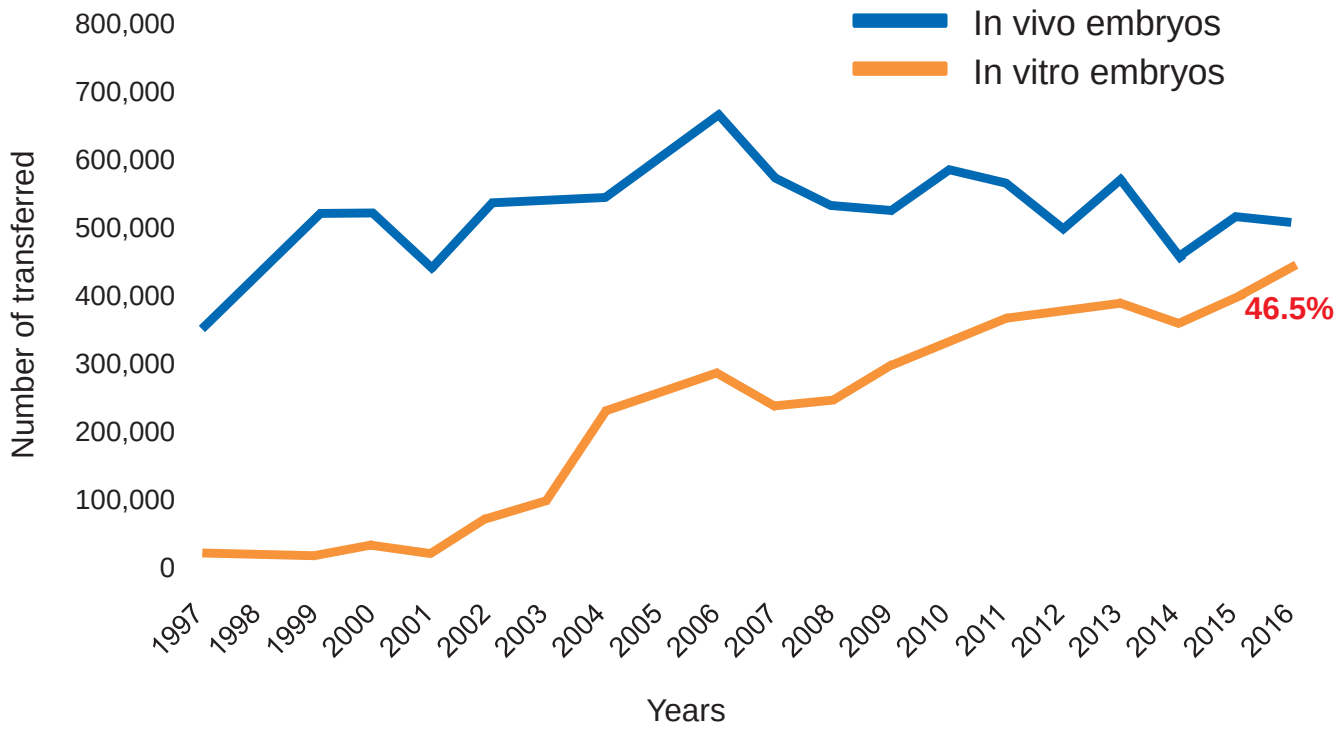
What is ovum pick-up (OPU)?

- An oocytes collection method from living donors
- A collecting method of the oocyte used for human assisted reproductive technology is applied to a cow.
- Observed the ovaries and aspirate the oocyte by needle with ultrasound image
- A probe and oocyte aspiration needle for cows are used.
- OPU is possible to collect oocytes once a week or twice a week.
- OPU is efficient embryo production system

Embryo transfer technologies

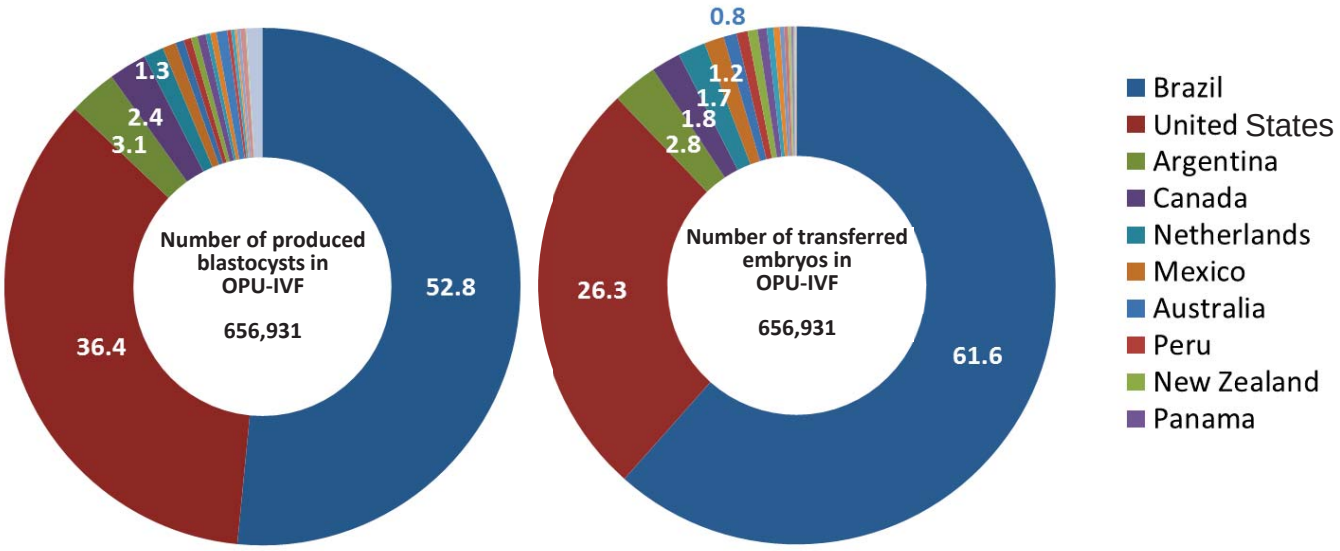


Changes in the number of transferred in *in vivo* and *in vitro* embryos in the world



Perry G, IETS data retrieval committee, Embryo Technology Newsletter 35(4):8-23, 2017

Bovine OPU-IVF in 2016



Increasing rates of OPU-IVF embryos in 2016

Regions	Increasing rates of produced and transferred OPU-IVF embryos (%)					
	Embryo production			Embryo transfer		
	Donors	Collected oocytes	Produced embryos	Fresh embryos	Frozen embryos	Total embryos
Africa	-44.4	-11.3	-42.0	134.0	1071.4	241.5
Asia	none	none	none	none	none	none
Europe	17.1	28.6	37.0	28.5	-16.3	12.8
North America	27.6	17.7	22.9	22.8	58.2	34.4
Oceania	36.1	23.1	62.0	159.3	-31.7	49.1
South America	-18.1	-5.6	2.3	2.8	10.9	4.5
Total	-2.3	0.8	8.7	7.7	22.9	11.4

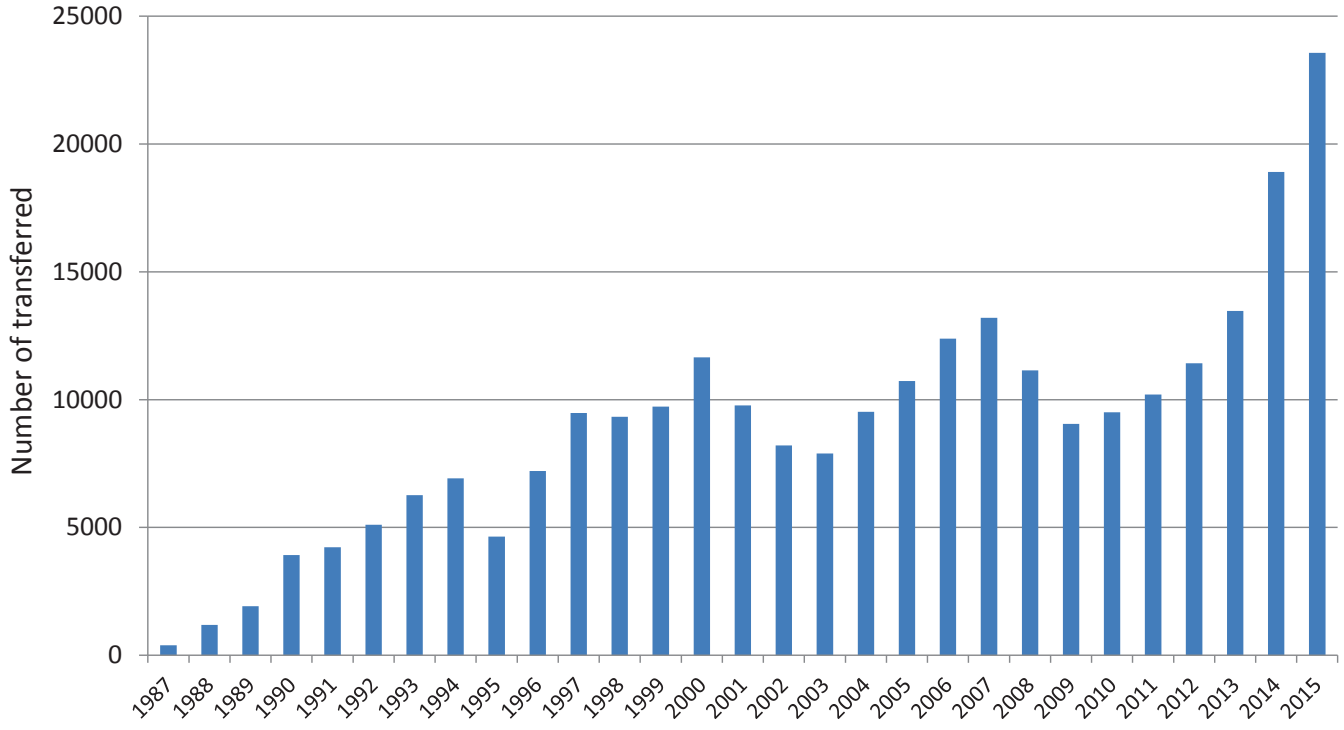
Perry G, IETS data retrieval committee, Embryo Technology Newsletter 35(4):8-23, 2017をもとに算出

Number of blastocysts/an OPU-IVF session

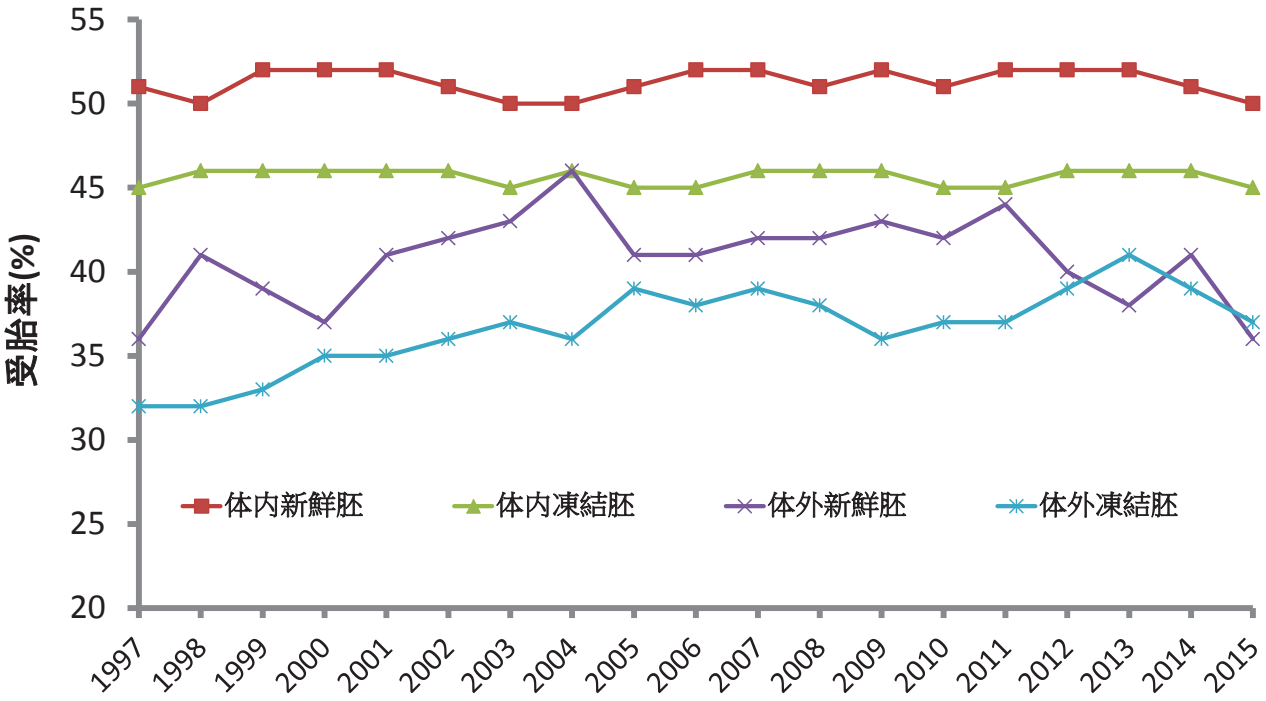
Name of countries	Treatments of donors											
	None						FSH stimulation					
	Dairy		Beef		Total		Dairy		Beef		Total	
	Oocytes	blastocysts	Oocytes	blastocysts	Oocytes	blastocysts	Oocytes	blastocysts	Oocytes	blastocysts	Oocytes	blastocysts
Brazil	25.5	8.9	25.5	8.9	25.5	8.9	--	--	--	--	--	--
USA	17.2	5.3	20.1	6.5	18.5	5.8	--	--	--	--	--	--
Argentina	46.3	17.1	21.9	6.5	22.0	6.6	--	--	--	--	--	--
Canada	--	--	--	--		--	9.8	4.1	10.9	5.5	9.8	4.2
Netherlands	--	--	--	--		--	9.7	1.8	--	--	9.7	1.8
Mexico	--	--	10.6	4.9	10.6	4.9	--	--	--	--	--	--
Peru	8.0	0.5	8.1	0.7	8.0	0.5	--	--	--	--	--	--
New Zealand	7.8	2.1	9.0	1.6	8.2	1.9	--	--	11.3	2.2	11.3	2.2
Panama	15.0	3.4	17.8	5.4	16.2	4.2	--	--	--	--	--	--
Germany	8.8	1.4	24.6	1.9	9.2	1.4	--	--	--	--	--	--
Spain	14.5	3.2	9.6	3.6	13.5	3.2	16.4	4.9	9.1	3.7	14.1	4.5
Dominican	19.2	5.0	27.4	8.3	22.1	6.2	--	--	--	--	--	--
France	--		--				8.3	2.4	17.9	8.9	8.7	2.7
Others	5.7	0.8	9.9	2.7	6.9	1.4	6.0	1.8	34.8	3.5	21.2	2.7
Total	18.6	5.8	22.1	7.4	20.1	6.5	9.5	2.7	25.4	4.1	10.8	2.9

Perry G, IETS data retrieval committee, Embryo Technology Newsletter 35(4):8-23, 2017

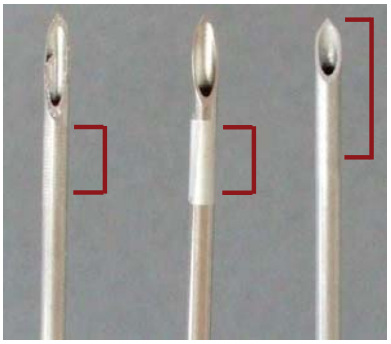
Numbers of transfer on IVP embryos in Japan



Changes in conception rates of IVP embryo in Japan



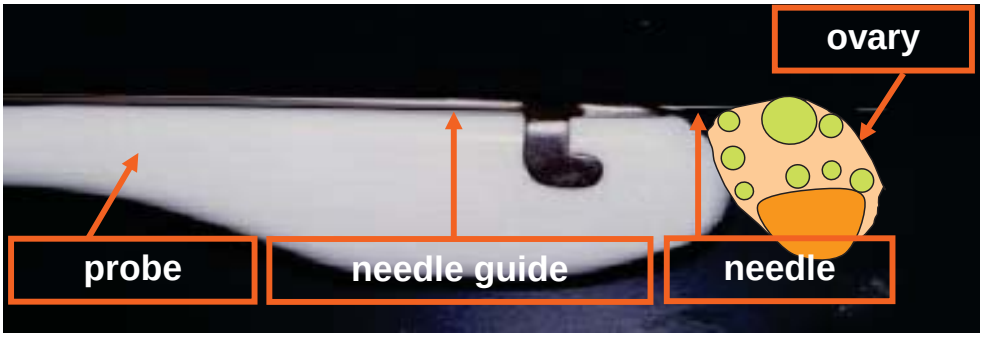
OPU system



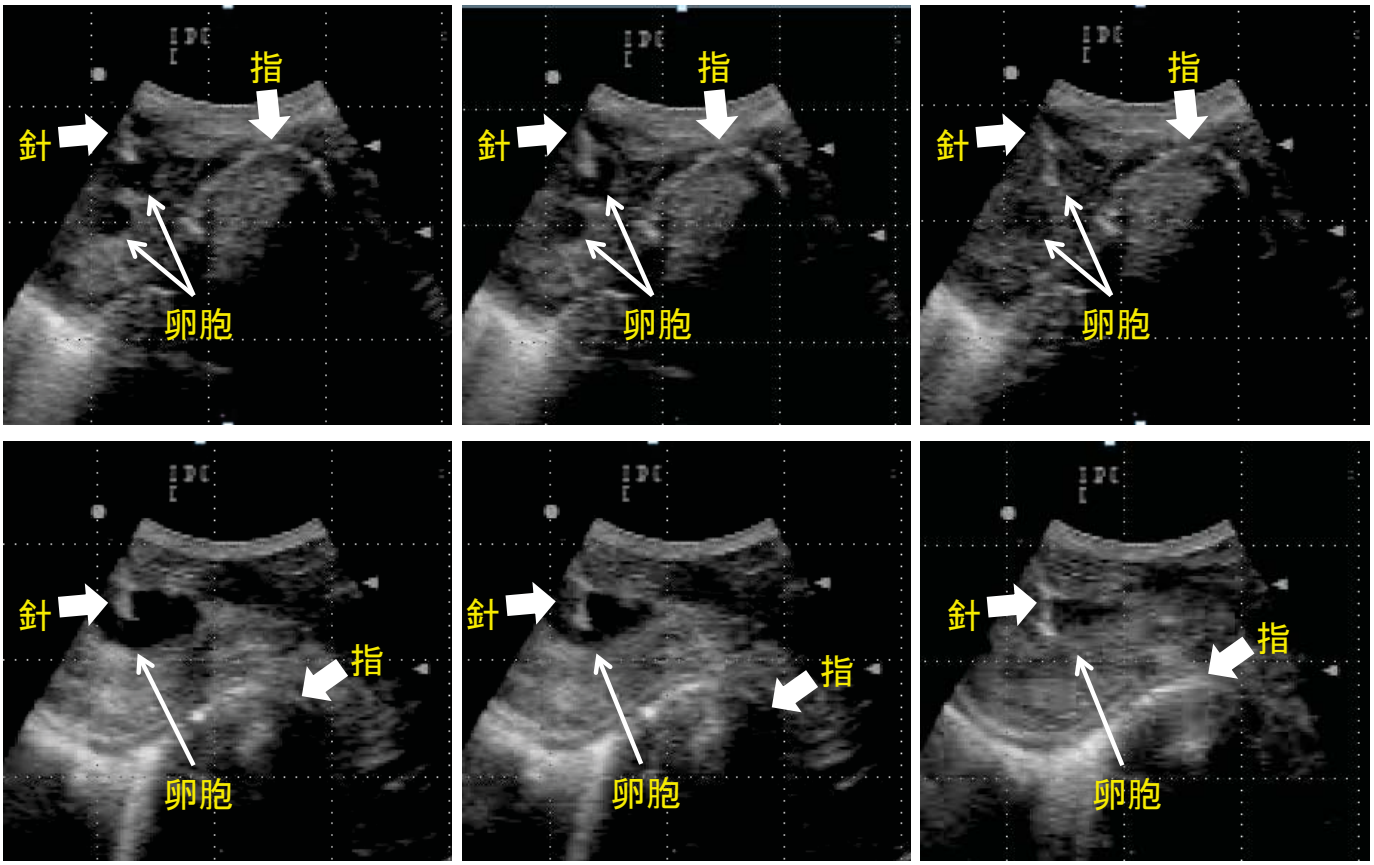
Double lumen | Single lumen | COVA needle

COVA needle

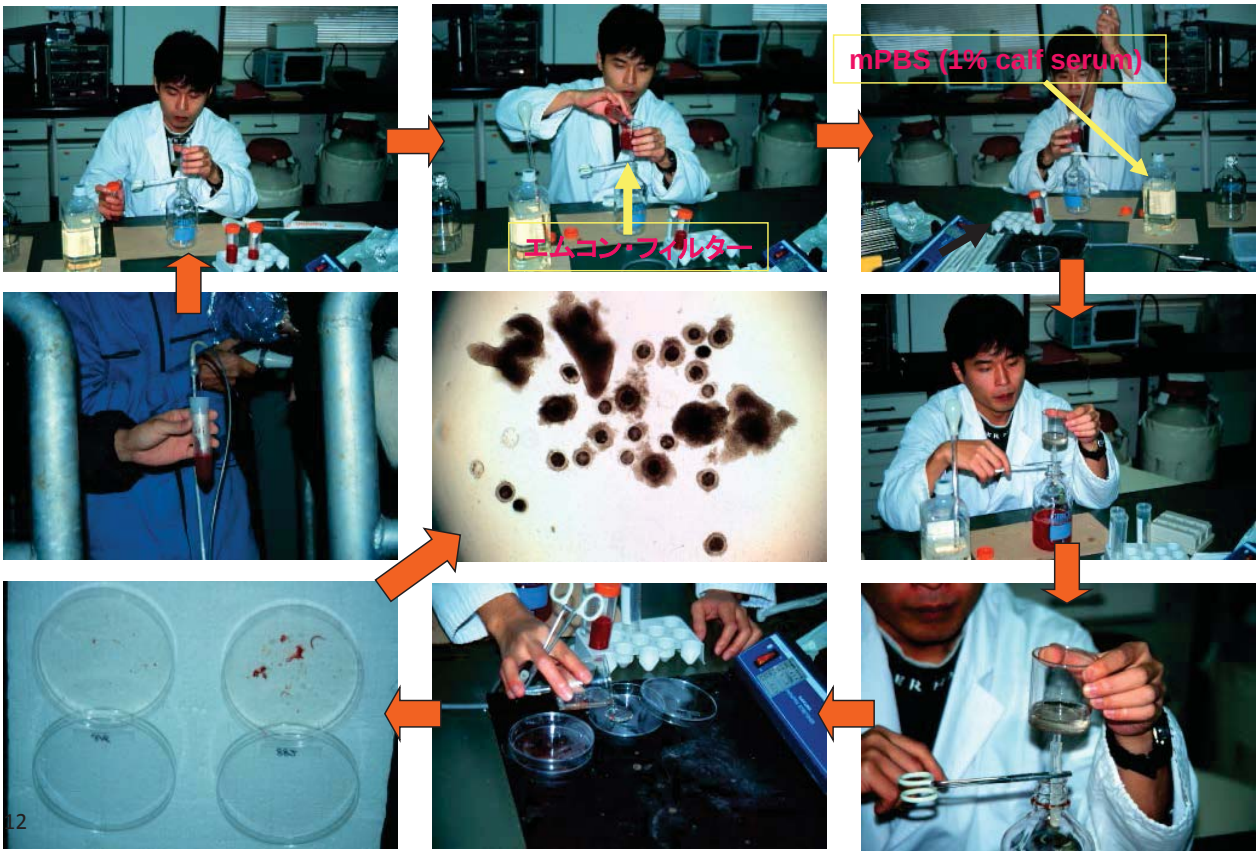
1. Obtuse angle
2. High visible
3. Silicon coated



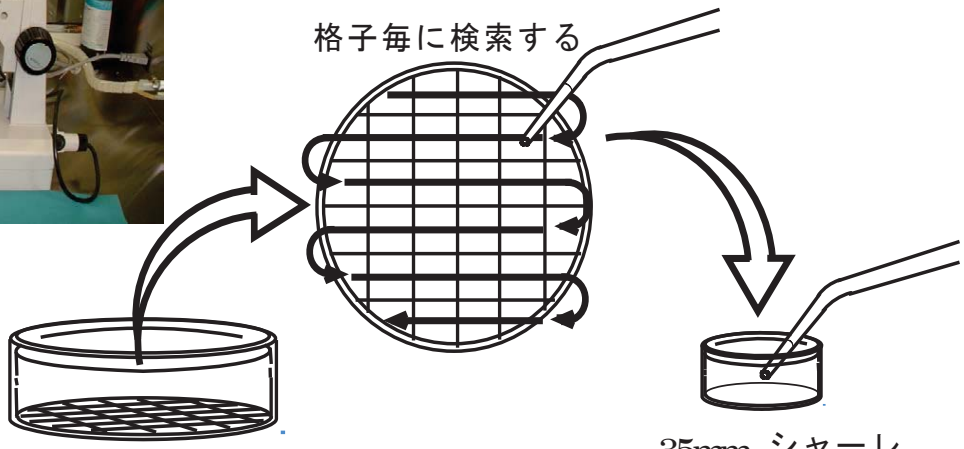
Oocyte aspiration from follicles



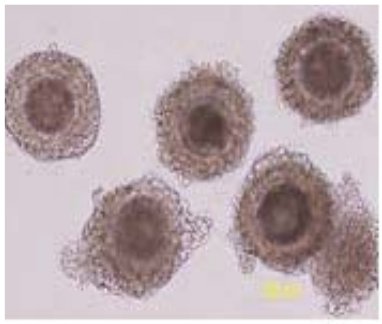
Filtration of aspirate fluid to clean up blood cells



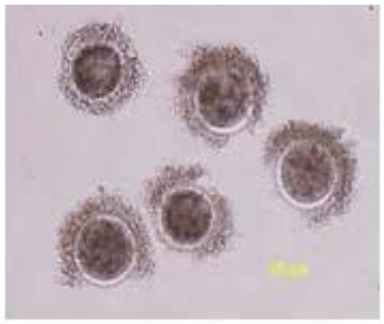
Search the aspirated oocytes



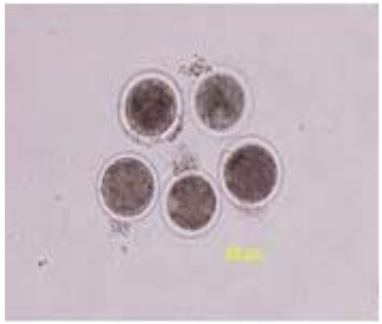
Evaluation of oocyte qualities



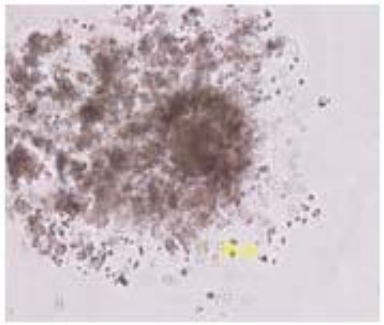
Grade 1



Grade 2



Grade 3



Grade 4

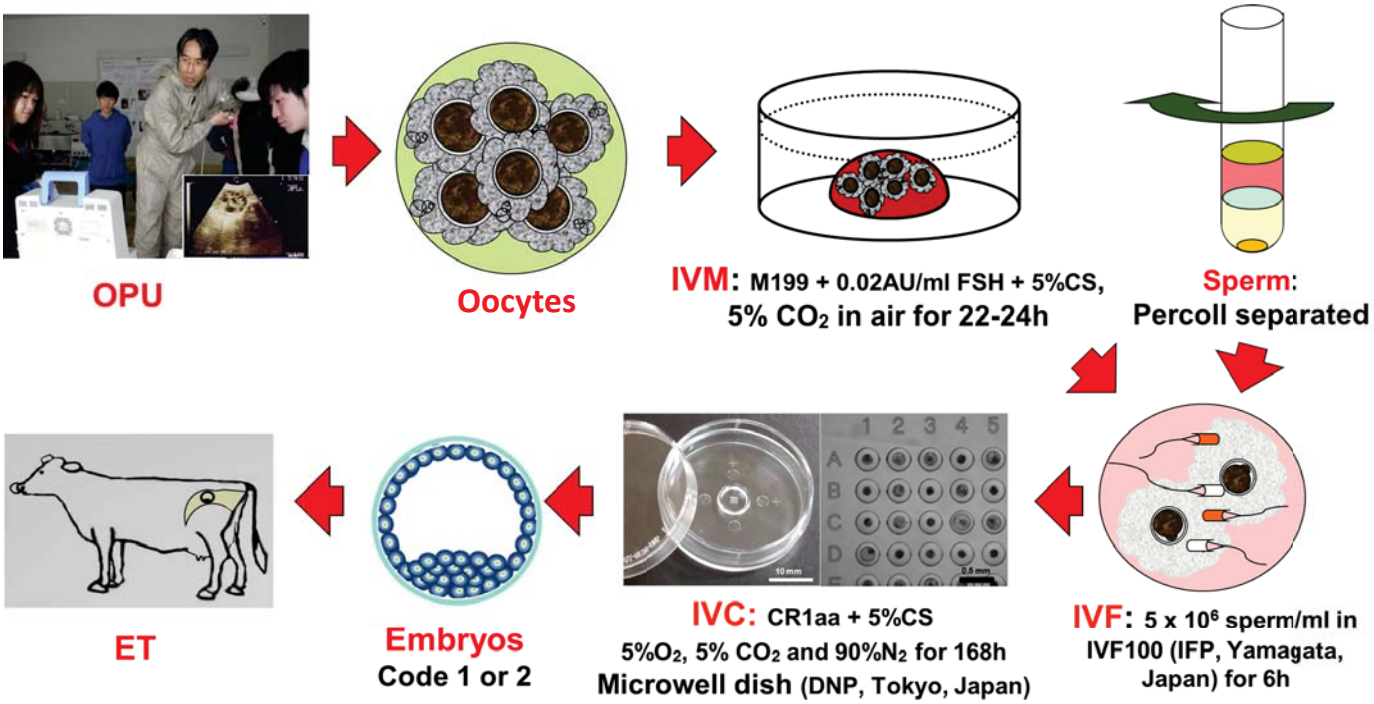
1: 3 or more layers of cumulus cell

2: less than 3 layers of cumulus cell or attached cumulus cell more than 1of 3 around zona pellucida

3: denuded oocyte or attached cumulus cell less than 1 of 3 around Zona pellucida

4: expanded cumulus cell

IVM-IVF-IVC method



Comparison of embryo production between MOET and OPU-IVF

Embryo production system	No. of OPU/MOET sessions	No. of follicles/CL	No. of collected oocytes/ova	No. of produced blastocysts
OPU-IVF	60	43.4 ± 16.4	36.7 ± 18.3	11.8 ± 7.6[a]
MOET	36	14.8 ± 9.8	9.3 ± 8.5	6.4 ± 6.3[b]

Values (means ± SD) within the same column with different superscripts differ (P < 0.01).

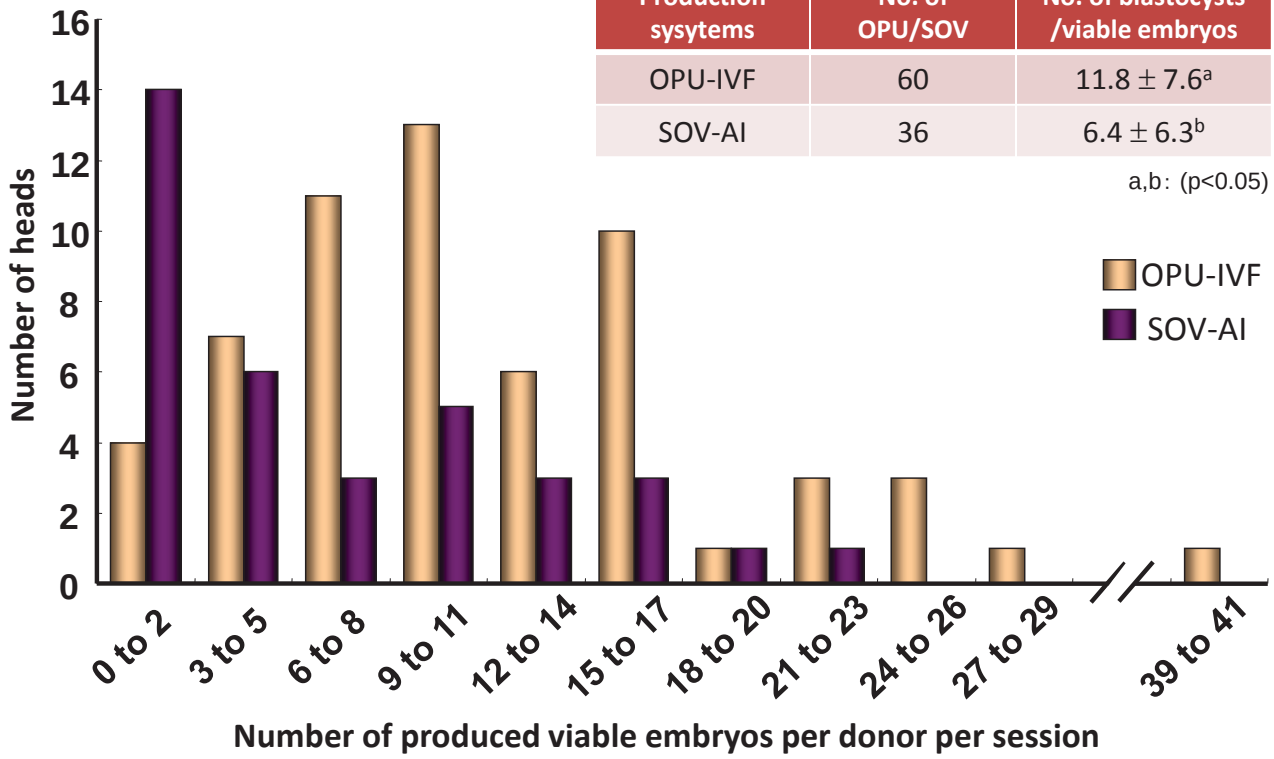
Data from more than 10 days OPU interval were collected.

Imai et al, J Reprod Dev 56(Suppl): S19-29, 2006

Comparison of embryo production between MOET and OPU-IVF

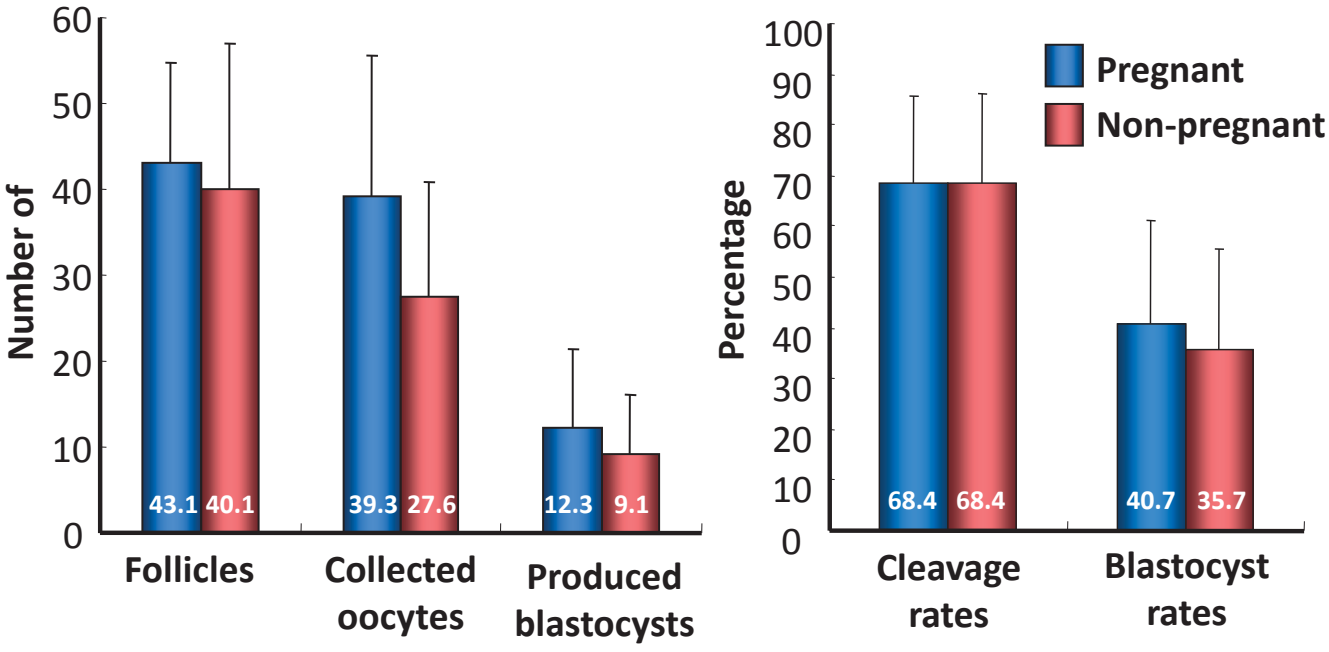
Production sysytems	No. of OPU/SOV	No. of blastocysts /viable embryos
OPU-IVF	60	11.8 ± 7.6[a]
SOV-AI	36	6.4 ± 6.3[b]

a,b: (p<0.05)



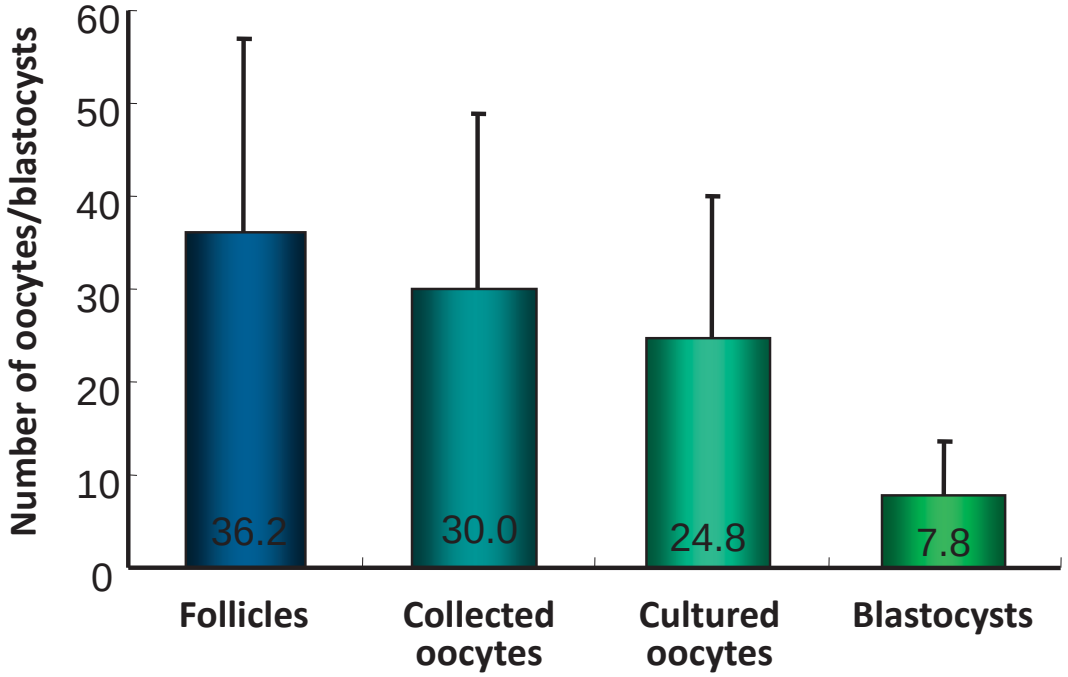
Imai et al, J Reprod Dev 56(Suppl): S19-29, 2006

Donors : Pregnant cows



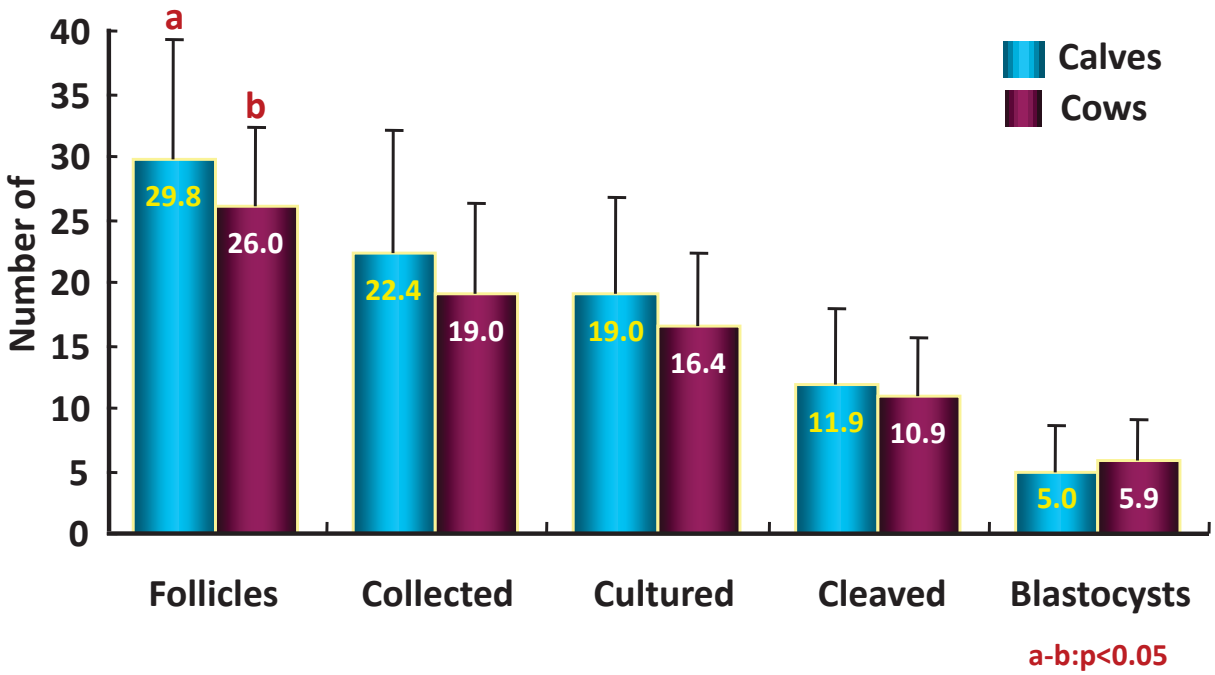
No significantly deference were found in all items

Donors: Reproductive disorders



Data from 17 OPU sessions

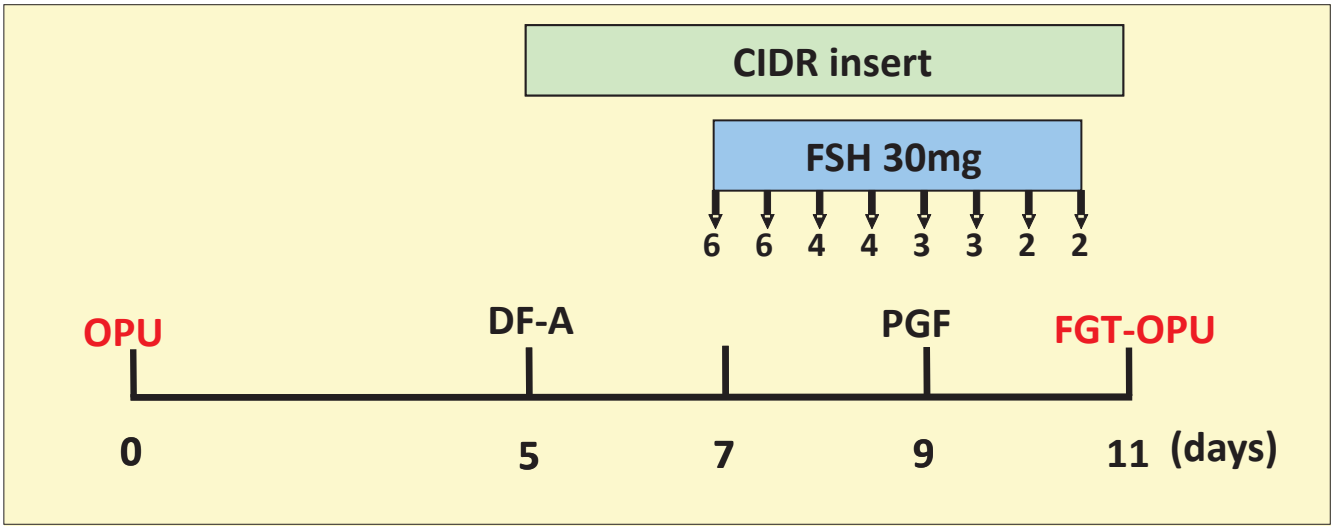
Donors: Calves (9 month, before puberty)



a-b:p<0.05

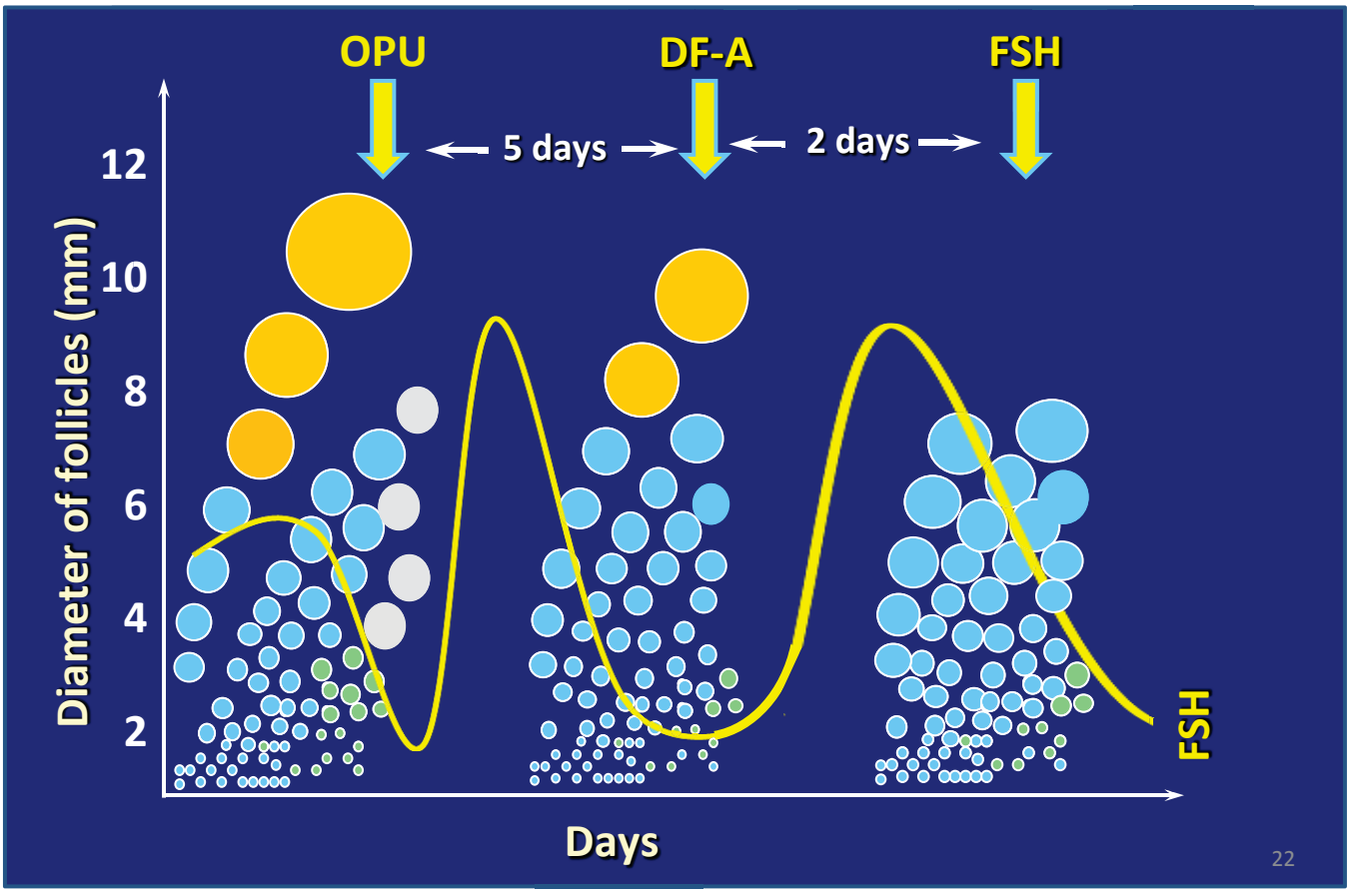
There was significant difference in the rates of blastocyst formation between calves (26.5%) and cows (36.0%).

OPU sessions after FGT

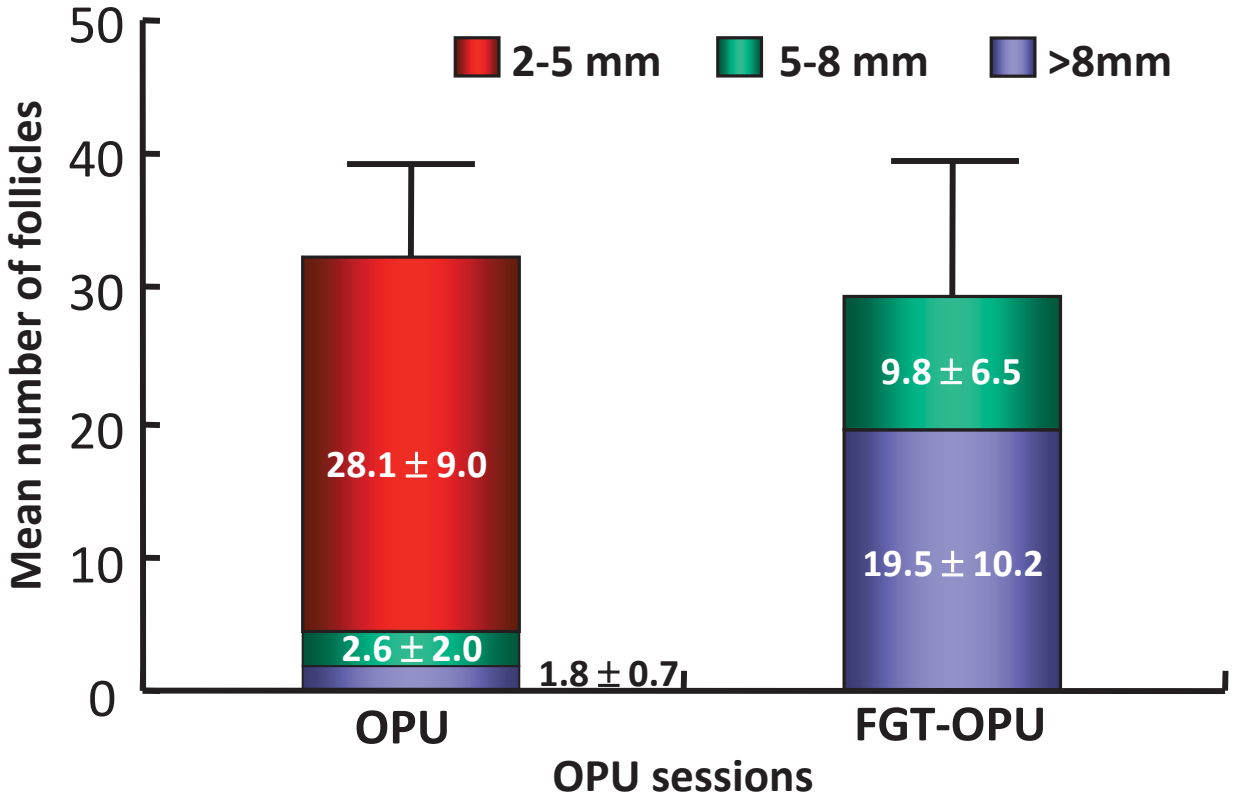


To assess the developmental competence of the oocytes collected during the first and second OPU sessions, the oocytes were examined to IVM-IVF-IVC.

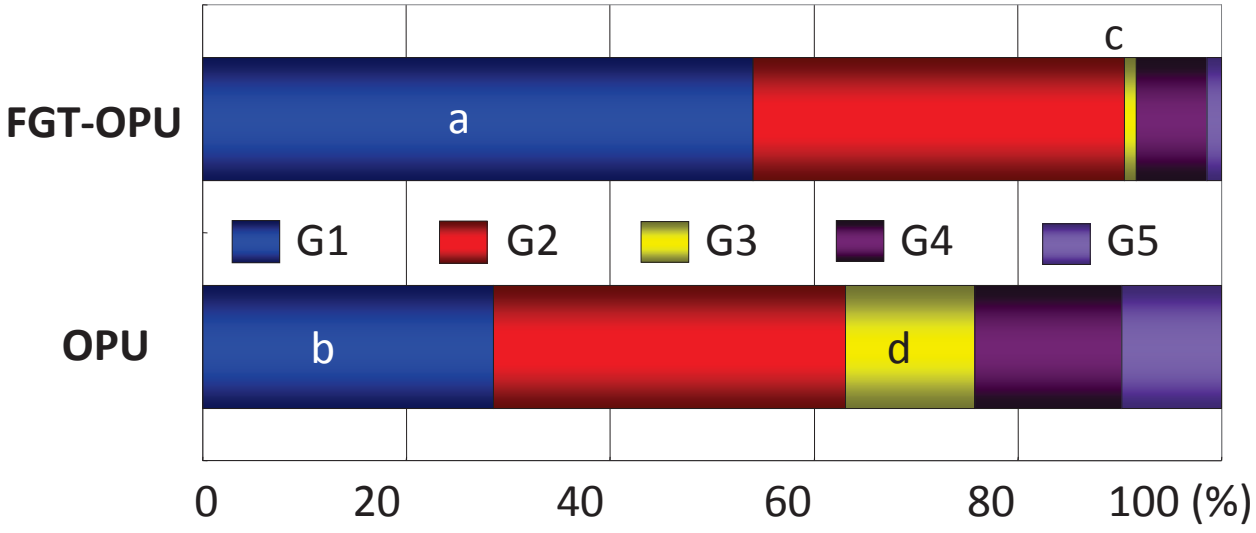
Follicular wave synchronization



Number of large, middle and small size follicles at the first and second OPU sessions



Quality of oocytes collected at the first and second OPU sessions



a,b; c,d: $p<0.01$

The percentage of G1 + G2 oocytes for the second OPU session (90.5 ± 13.8) was significantly higher than that for the first OPU session (63.1 ± 6.3).

Results of embryo production at the first and second OPU sessions

Number/OPU session	OPU	FGT-OPU
Follicles	32.5 ± 6.8	29.3 ± 10.4
Oocytes collected	26.0 ± 12.7	19.0 ± 9.4
Oocytes inseminated	20.8 ± 10.1	18.1 ± 9.6
Oocytes cleaved	13.1 ± 7.9	14.8 ± 9.0
Blastocysts	4.3 ± 2.9[a]	12.8 ± 8.7[b]

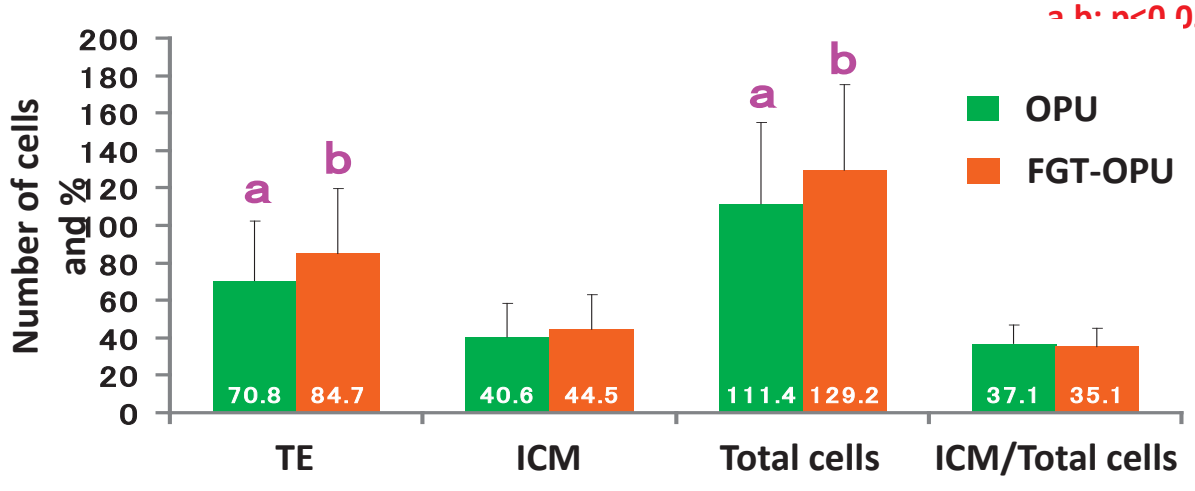
a,b : p<0.05

Oocytes derived from FGT-OPU

In vitro fertilization of FGT-OPU

Session	Oocytes	Matured (%)	Penetrated (%)	2 pronuclei	3 pronuclei	> 4 pronuclei
OPU	86	72 (83.7)	71 (82.6)	64.8[b]	22.5	12.7
FGT-OPU	85	70 (81.4)	67 (77.9)	83.6[a]	11.9	4.5

a,b: p<0.05



a,b : p<0.01

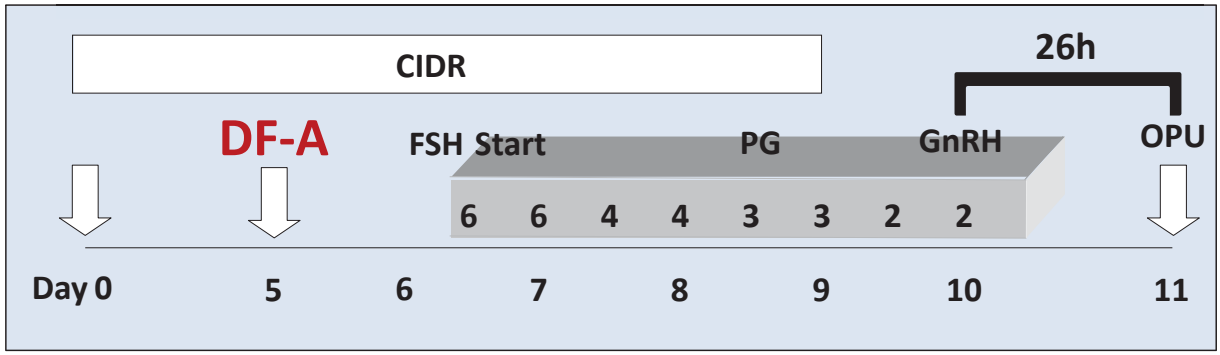
Imai et al, IETS meeting (2010)

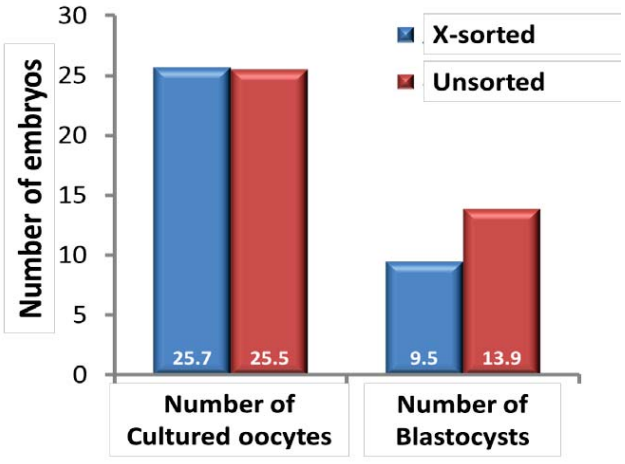
Conception rate of FGT-OPU-IVF embryos

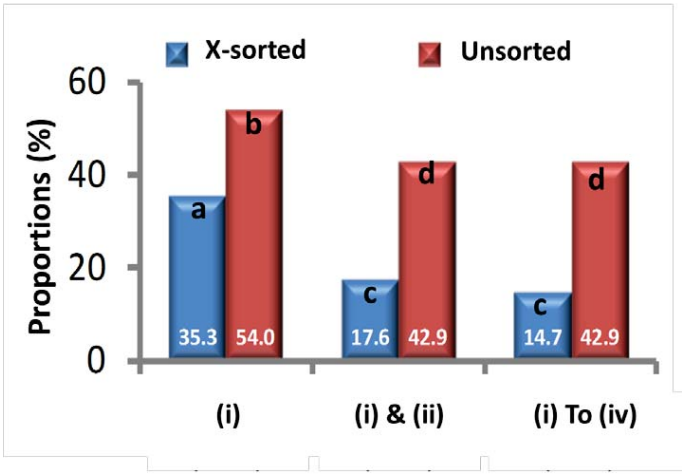
Sessions	No. of recipients	No. of conceptions (%)
OPU	29	12 (41.4)
FGT-OPU	16	9 (56.3)

Imai et al, unpublished data

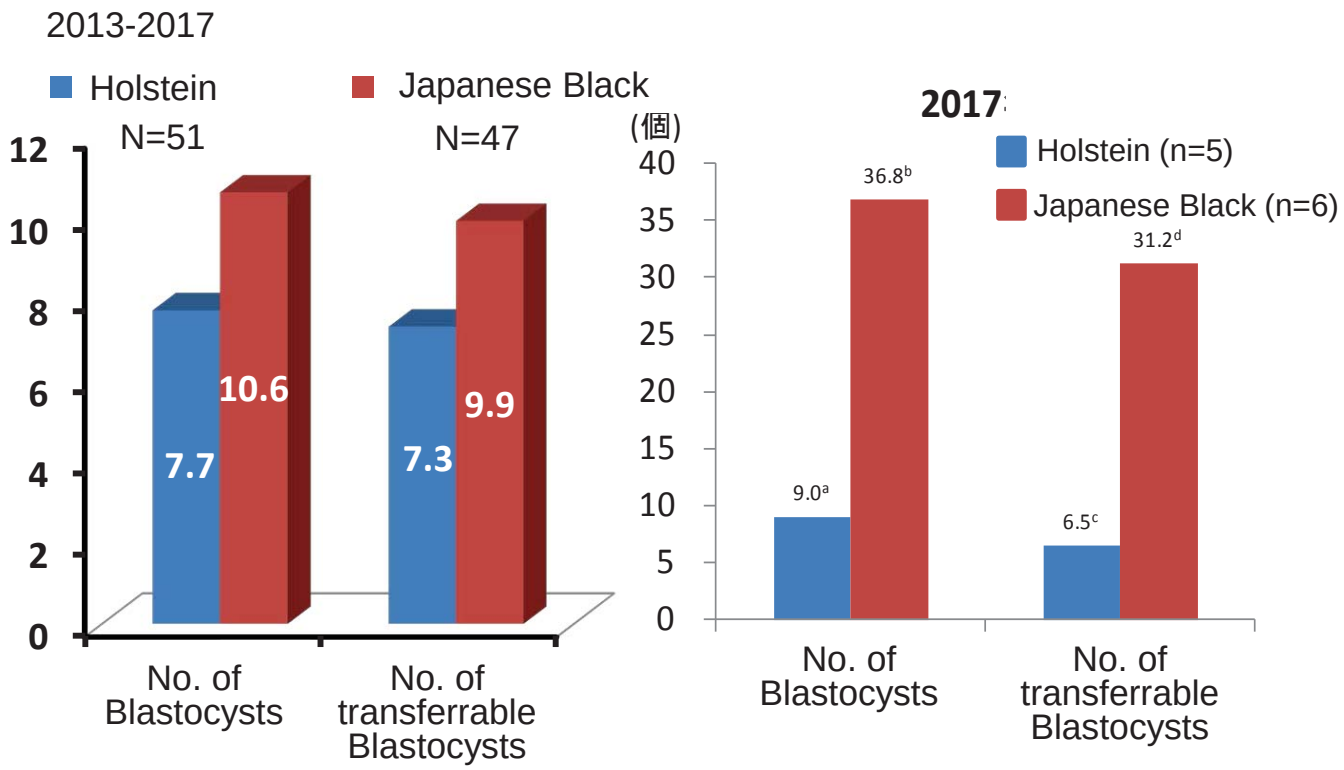
Collect in vivo matured oocytes







Results of embryo production byOPU-IVF in Rakugo Gakuen University



Embryo production by OPU-IVF in various donors

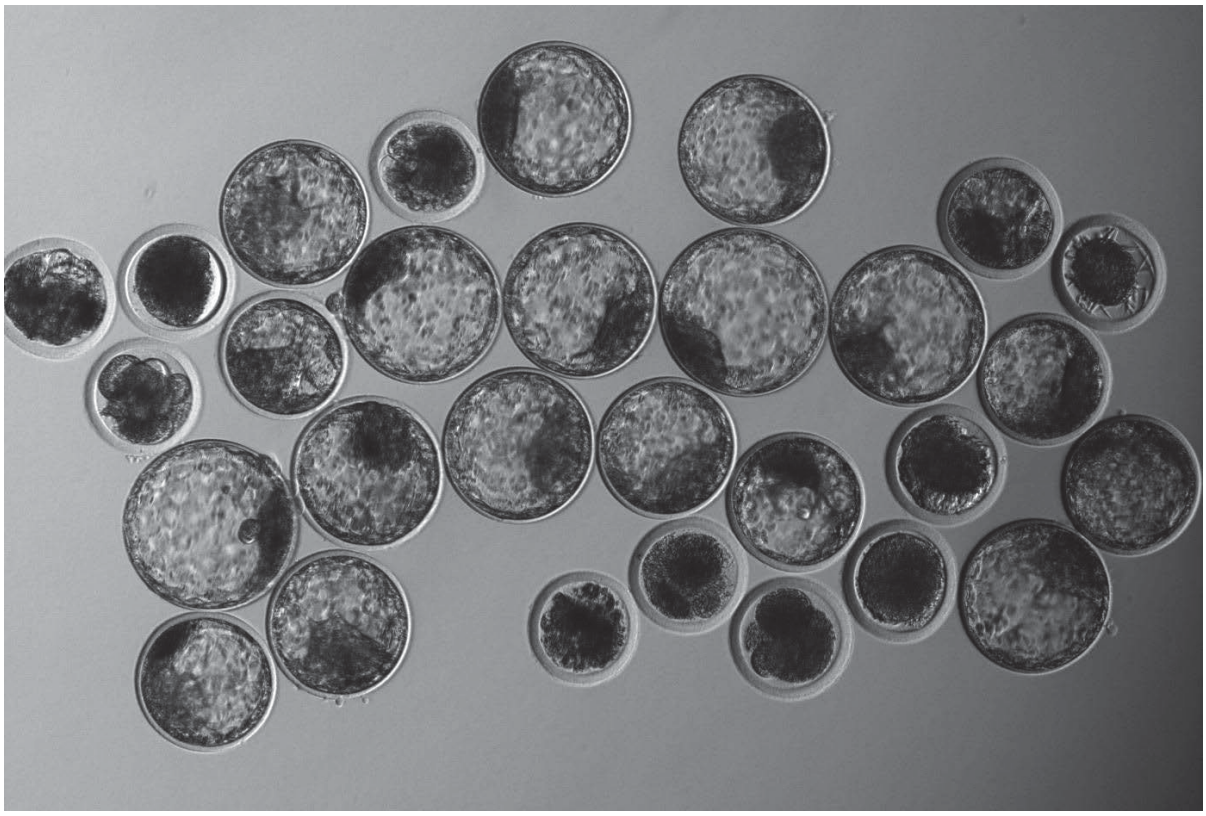
Donors	No. OPU sessions (intervals)	No. follicles	No. oocytes/ OPU session	No. blastocysts/ OPU session	% blastocyst
Dry cows	60 (>10)	43.4	36.7	11.8	41.6
Pregnant cows	16 (7)	43.1	39.3	12.3	40.7
Reproductive disorder	17 (>10)	36.2	30.0	7.8	29.3
9 months calves	28 (7)	29.8	22.4	5.0	26.0
6 months calves	2 (7)	45.0	32.5	5.0	19.5
FGT treatment	8 (11)	29.3	19.0	12.8	68.1
SOV treatment	8 (>10)	45.6	25.5	13.9	57.2

Summary: embryo production by OPU-IVF

The efficiency of embryo production by OPU-IVF

- OPU-IVF is more efficient embryo production system than MOET.
- There is no difference in the reproductive status, if ovaries have enough number of follicles.
- Small follicle < large follicle =/< in vivo matured
- By improving the donor treatments, we were able to produce more than 13 blastocysts per donor cow per session by IVF of OPU-derived in vivo mature oocytes.
- However, there are some problems for embryo quality of blastocysts derived from x-sorted semen.

Which embryos have viability of pregnancy?



Background of selection system

Compare in vitro and in vivo produced embryos

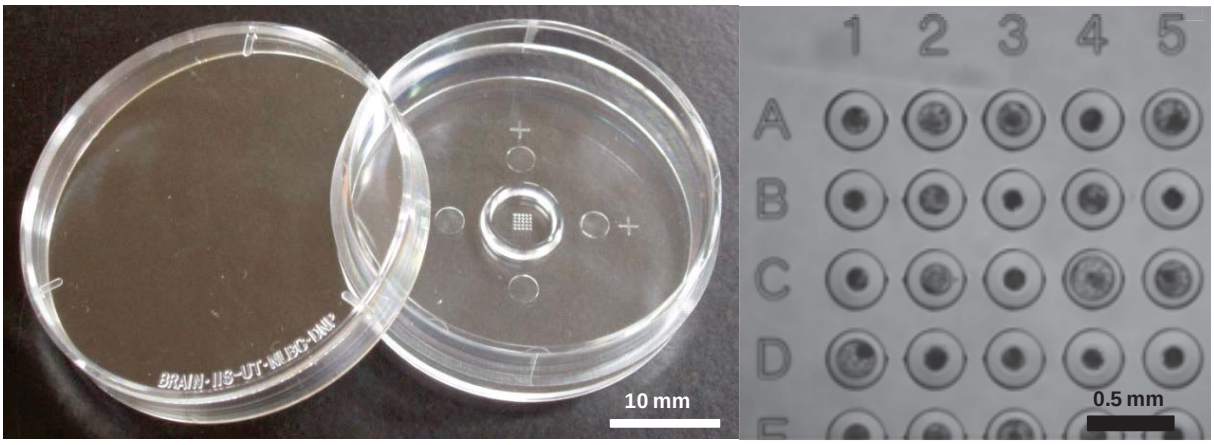
- Low conception rate
- Increase birth weight (large offspring syndrome)
- Extend gestation period
- Low cryotolerance

New technology for in vitro culture

- Individual culture system for IVF embryo
- Time-lapse cinematography （TLC)
- Oxygen consumption of embryo by scanning electrochemical microscope (SECM)

To select the healthy and high viability embryos by TLC, we conducted to relationship between the kinetics of embryo development and their quality.

Individual culture in IVF embryo



WOW dish : 35mm culture dish
well : 7mm in diameter at the center of dish
25 microwells (280μm in diameter and 160μm in depth, taper=7°)

Culture : 125μl of medium, 25 embryos
5%O_2, 5%CO_2, 90%N_2 and saturated humidity

Sugimura et al, Biol Reprod, 2010

Monitoring of embryo development by time-lapse cinematography (TLC)

Real-time culture cell monitoring system (Astec, Fukuoka, Japan)

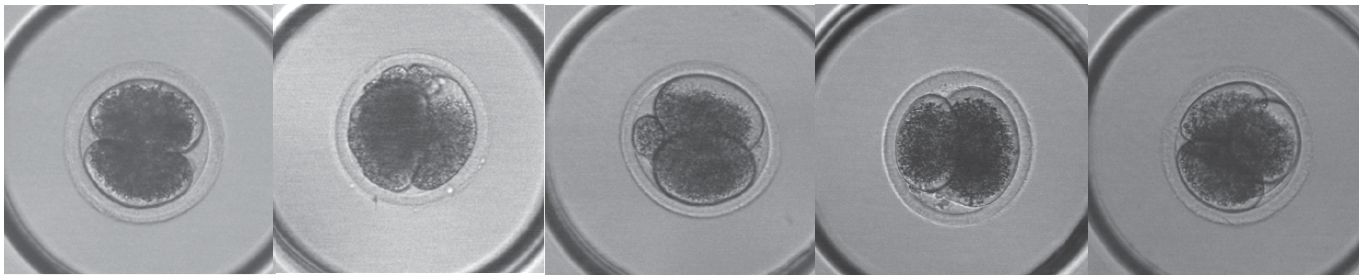
TLC: It take photos every 15min, then take 673 photos until finish the culture for 7 days

第1卵割の形態による受胎および分娩

Morphology of 1st cell cycle		Transfer	Pregnancy (%)	Delivery (%)
Normal	NC	29	15 (51.8)[a]	14 (48.3)
Abnormal	MF	3	0 (0.0)	0 (0.0)
	PT*	3	1 (33.3)	1 (33.3)
	UB*	6	2 (33.3)	2 (33.3)
	3-4BL	18	5 (27.7)	5 (27.7)
	計	29	7 (24.1)[b]	7 (24.1)

＊同一の受胎した胚1個を含む

a,b: p<0.05



Normal Cleavage (NC) | Multiple Fragments (MF) | Protrusion (PT) | Uneven Blastomeres (UB) | 3-4 Blastomeres (3-4BL)

Five prognostic factors for embryo quality

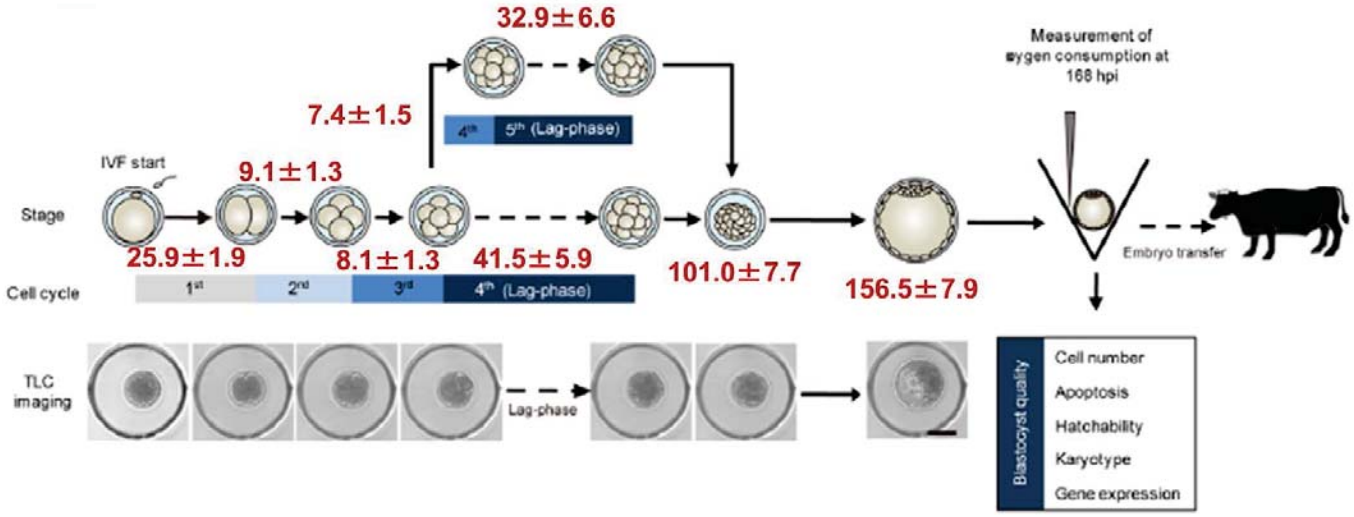
PF1: Timing at 1st cell cycle

PF2: Number of blastomeres after 1st cell cycle

PF3: Existence of fragments

PF4: Number of blastomeres at lag-phase

PF5: Oxygen consumption of blastocyst



Sugimura et al, PLoS ONE (2012)

Kinetics of embryo development affect the normality of chromosomes

	End of first cleavage			Onset of lag-phase	
Timing	Diplodi blastocyst (%)	Number of blastomere	Diploid blastocyst (%)	Number of blastomere	Diploid blastocyst (%)
Fast <27 hpi	58/86 (67.4)	2	55/72 (76.4)	4-5	6/13 (46.2)
				6-8	22/24 (91.7)
				9-16	27/35 (77.1)
		3-4	3/14 (21.4)	4-5	0/1 (0)
				6-8	1/2 (50.0)
				8-16	2/11 (18.2)
Slow ≥27 hpi	5/25 (20.0)	2	5/16 (31.3)	4-5	1/5 (20.0)
				6-8	3/9 (33.3)
				8-16	1/2 (50)
		3-4	0/9 (0)	4-5	0/0 (0)
				6-8	0/2 (0)
				8-16	0/7 (0)

Sugimura et al, PLoS ONE, 2012

Conception rates in IVF embryos selected by prognostic factors

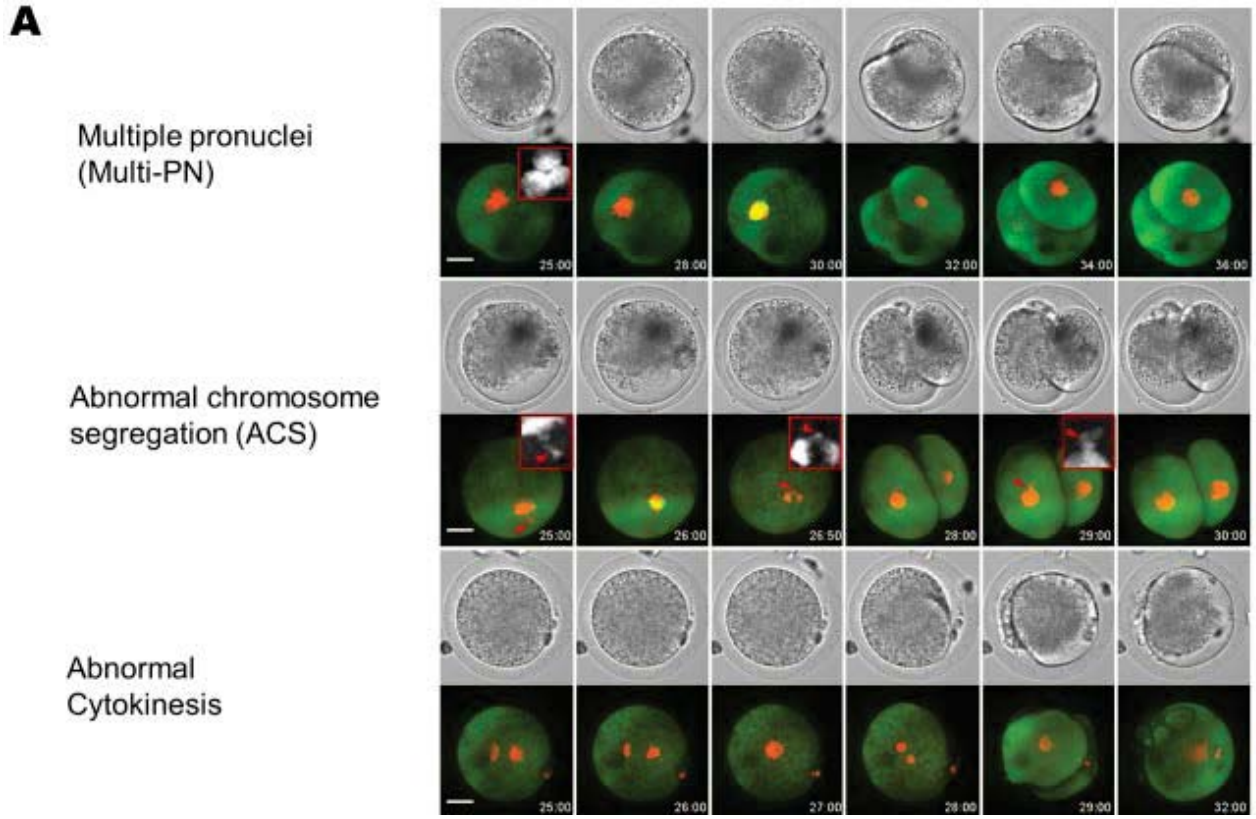
Combinations	No. of transferred	No. of conceptions	% of conception
Conventional method	52	21	40.4
PF1 and PF2	27	18	66.7*
PF1, PF2, and PF3	24	17	70.8*
PF1, PF2, PF3, and PF4	22	16	72.7*
PF1, PF2, PF3, PF4, and PF5	19	15	78.9**

Compared with conventional method: * $p<0.05$, ** $p<0.01$

The birth weight was 29.2 ± 3.3 kg that was close to that derived from AI embryos (28.7 ± 4.2 kg) and we observed no neonatal overgrowth or death.

Sugimura et al, PLoS ONE (2012)

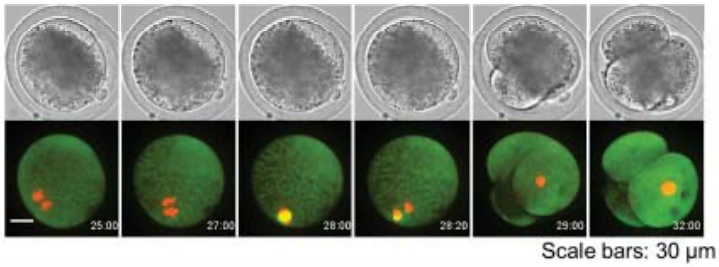
Live cell imaging of abnormal cell division



Tatsuma Yao, Sci Report 8:7460, 2018

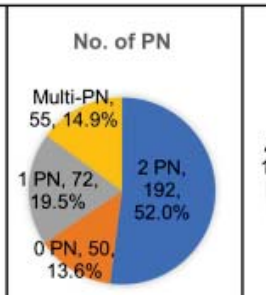
Live cell imaging of abnormal cell division

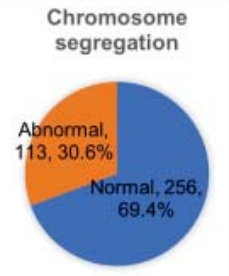
Multiple blastomeres after first cleavage (Multi-division)

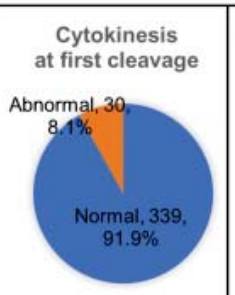


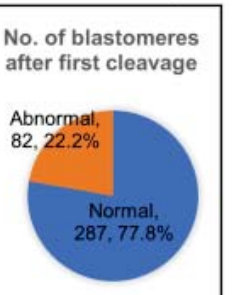
Scale bars: 30 μm

B





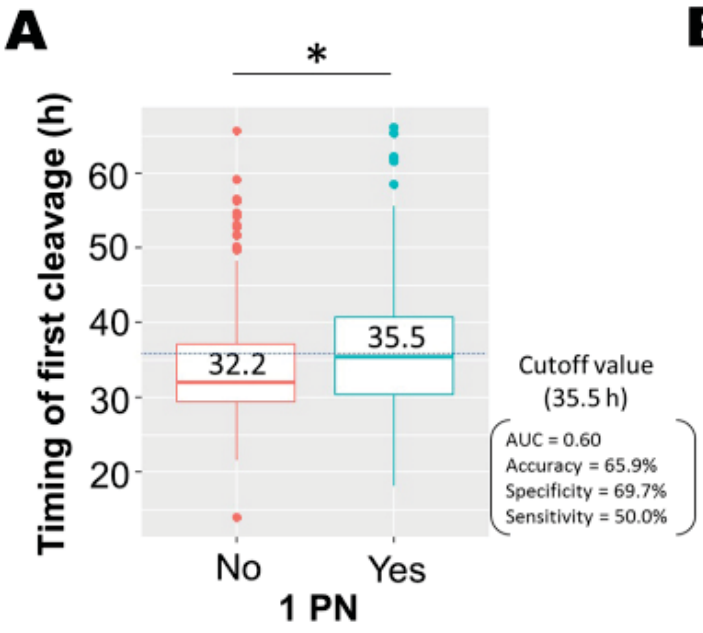


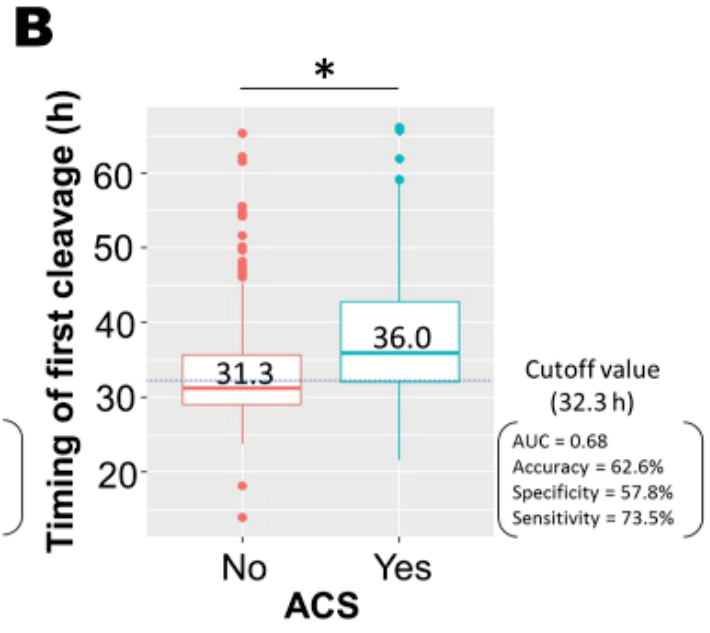


	No. of PN		Chromosome segregation		Cytokinesis at first cleavage		No. of blastomeres after first cleavage	
	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal
8-cell (%)	58/177 (32.8%)	86/192 (44.8%)	18/113 (15.9%)	126/256 (49.2%)	1/30 (3.3%)	143/339 (42.2%)	28/82 (34.1%)	116/287 (40.4%)
Morula (%)	41/177 (23.2%)	66/192 (34.4%)	10/113 (8.8%)	97/256 (37.9%)	1/30 (3.3%)	106/339 (31.3%)	18/82 (22.0%)	89/287 (31.0%)
Blastocyst (%)	23/177 (13.0%)	43/192 (22.4%)	7/113 (6.2%)	59/256 (23.0%)	1/30 (3.3%)	65/339 (19.2%)	11/82 (13.4%)	55/287 (19.2%)

Tatsuma Yao, Sci Report 8:7460, 2018

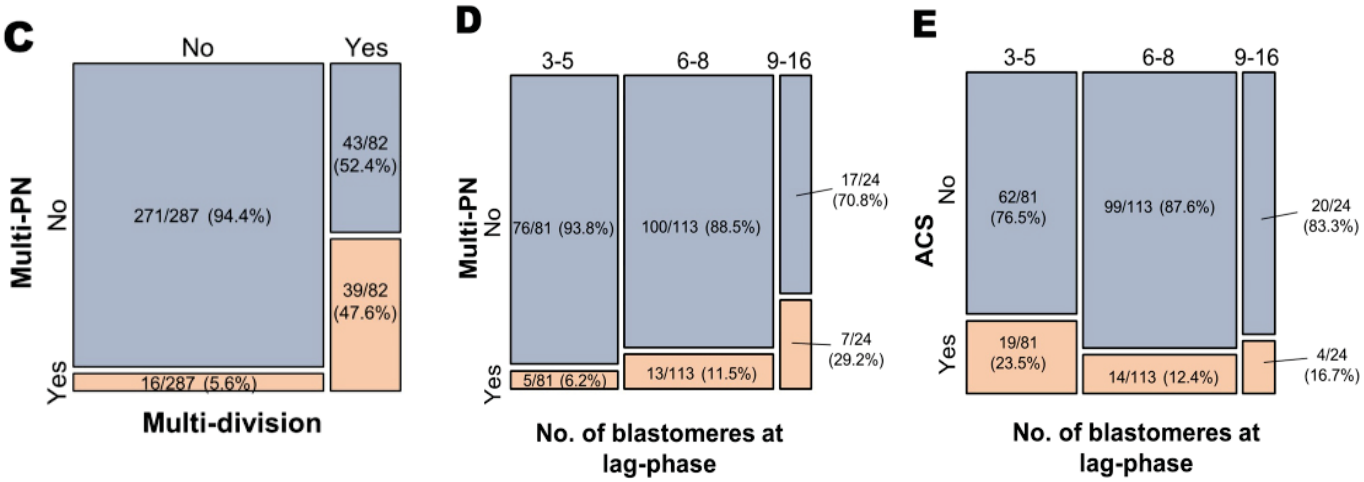
Effect of abnormal cell division on timing of 1st cleavage





Tatsuma Yao, Sci Report 8:7460, 2018

A hierarchical relationship between more than multi-division and multi-PN

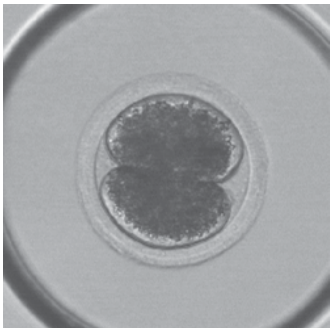


Tatsuma Yao, Sci Report 8:7460, 2018

Summary: Selection of embryos

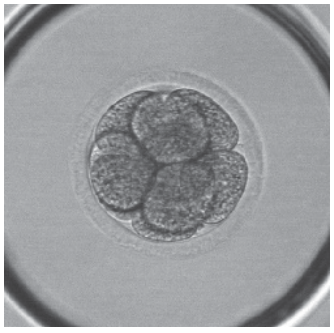
Time-Lapse Cinematography

1st cell cycle



Less than 27 hpi
Two blastomeres
Without fragments

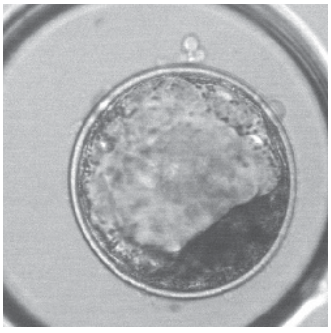
Lag-phase



Six blastomeres or more
Without fragment

Oxygen consumption

Blastocyst



High oxygen consumption

IVF embryos: high competence of conception and delivery

Time-lapse cinematography could be used great tool for quality assessment of IVF bovine embryos.

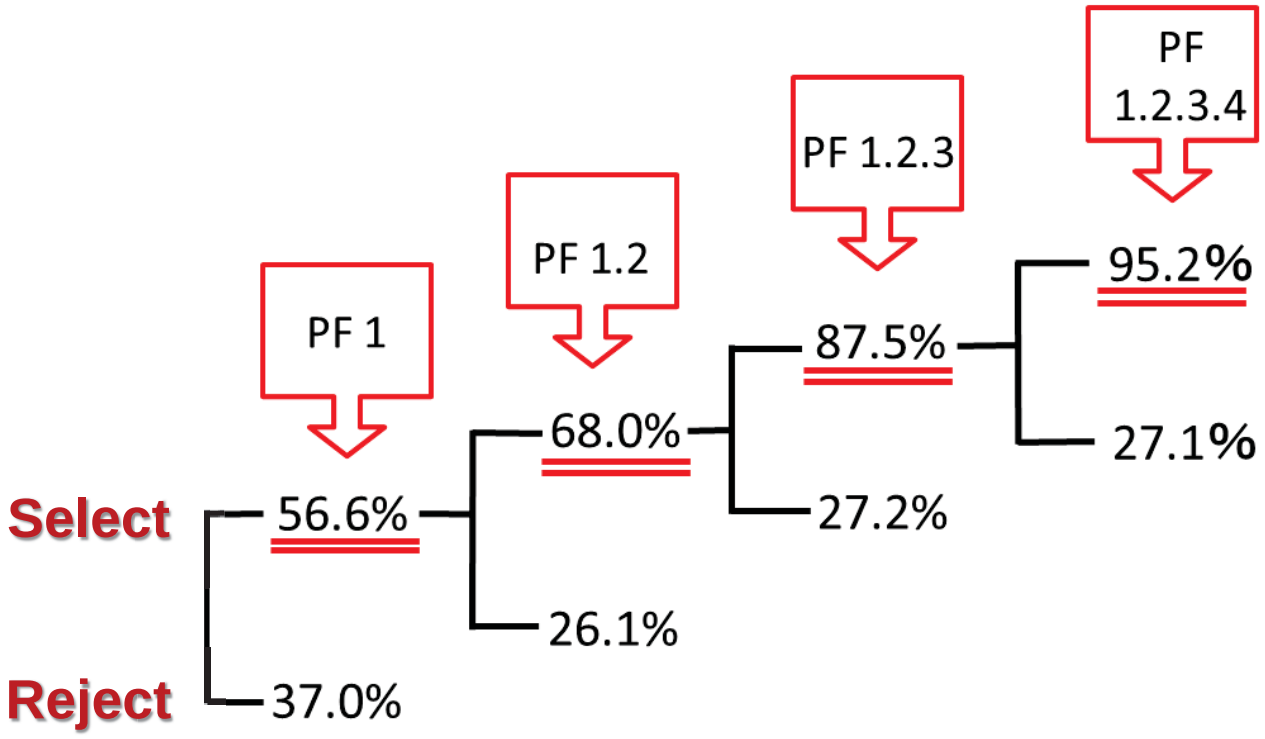
Embryo selection system with four factors in the three observations

To select healthy embryos, a combination of four factors were used as follows:

In case of the first cleavage is earlier (more than 50% cleaved embryos were found until 27 hpi)
①Timing at first cleavage (less than 27 hpi)
②Two blastomeres after first cleavage
③Absence of fragments after first cleavage at 31 hpi
④Eight or more blastomeres at 55 hpi

In case of the first cleavage is slower (more than 50% cleaved embryos were found from 27 to 31 hpi)
①Timing at first cleavage (less than 31 hpi)
②Two blastomeres after first cleavage
③Absence of fragments after first cleavage at 31 hpi
④Eight or more blastomeres at 55 hpi

Effect of prognostic factors in embryo development



Taniai et al., IETS meeting, 2015

Pregnancy rates of selection system

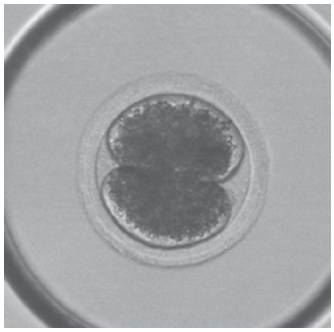
Morphological	No. transfer	No. pregnant	Proportions (%)
Fair	21	12	57.1
good	24	13	54.2

PF 1 to 4	No. transfer	No. pregnant	Proportions (%)
Select	23	17	73.9[a]
Reject	22	8	36.4[b]
Reject 1PF	14	6	42.9
Reject 2PFs	8	2	25.0
Total	45	25	55.6

Takayama et al.,2016 in ICAR

In case of no TLC and oxygen consumption

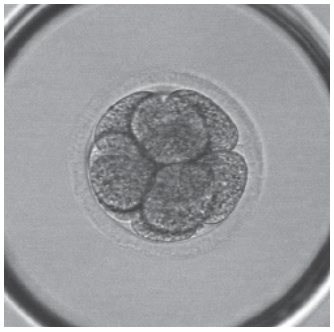
1st cell cycle



Observation of cleavage 27h & 31h after insemination

2 blastomeres & no fragments, equal volume

Lag-phase



Observation of cleavage 51h after insemination

6-16 blastomeres & no fragments

Possible to get more than 70% pregnancy rate

Conclusions

1. Embryo production by OPU-IVF is efficient and stable
2. Individual culture and TLC
3. Selection of embryos with high competence of pregnancy
4. Prognostic factors can be useful for analyze sperm and oocyte quality

Improve the embryo quality, calf production rate and efficient utilization of recipients

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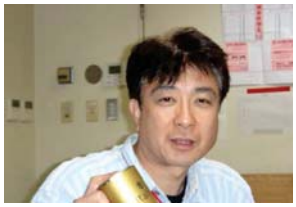

Thank you for your attention !

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