

## Bovine embryo production and selection system using OPU-IVF and kinetics early embryo development

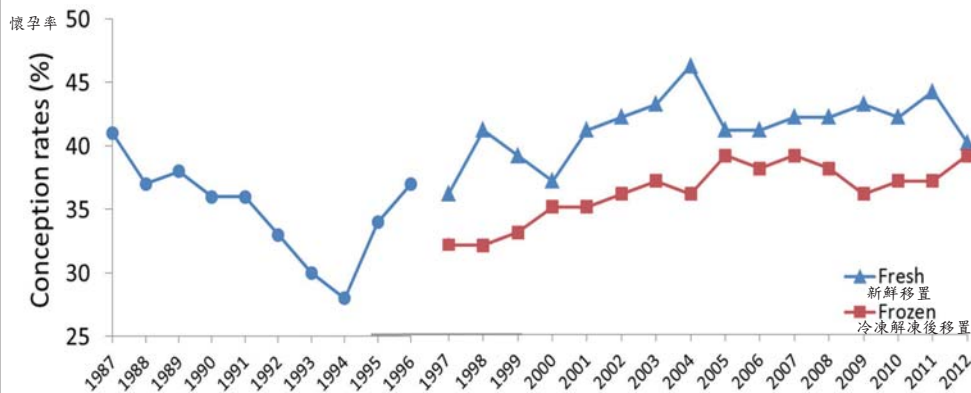
使用活體採卵與體外受精系統生產之牛胚早期品質鑑定系統與胚胎發育動力學  
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## Conception rates of IVP embryo in Japan

日本IVF牛胚胚移置懷孕率



## Transfers of Bovine IVP embryos in 2013

2013年牛體外受精胚的胚移置數量統計

Resions (地區)	IVP embryos (體外胚)	Transfer of IVP embryos (胚移置數)		
		Fresh (新鮮)	Frozen (冷凍)	計(%)
Africa (非洲)	5,012	3,101	1,562 (33.5)	4,663 (1.1)
N. America (北美)	114,393	55,912	13,121 (19.0)	69,033 (16.8)
S. America (南美)	376,459	289,903	15,025 (4.9)	304,928 (74.3)
Asia (亞洲)	30,067	8,571	6,422 (42.8)	14,993 (3.7)
Europa (歐洲)	14,772	9,281	2,833 (22.4)	12,114 (3.0)
Oceania (大洋洲)	5,925	2,426	3,041 (55.6)	5,467 (1.3)
total (合計)	546,628	369,194	42,004 (10.2)	411,198 (100)

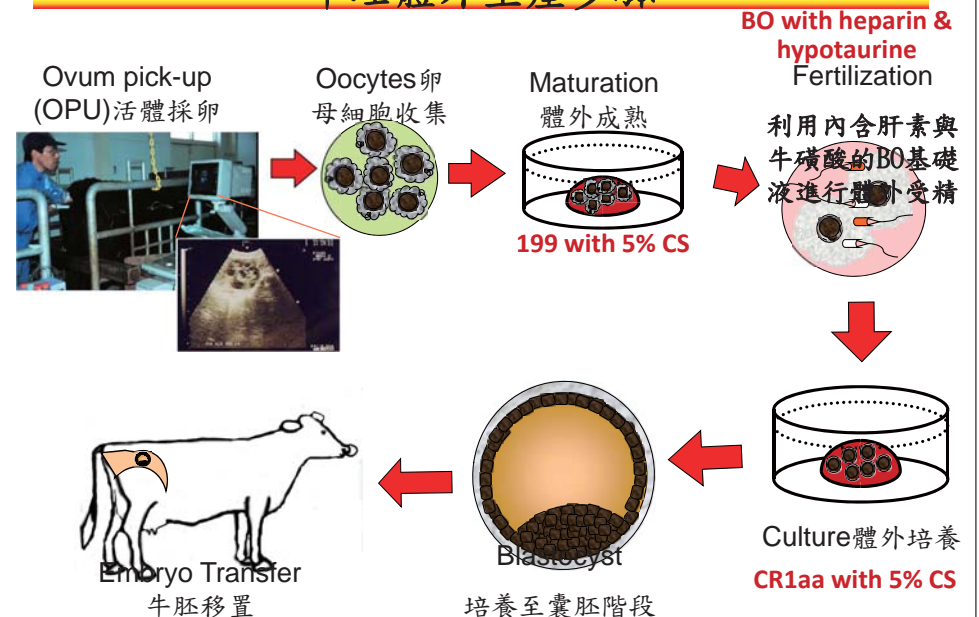
Embryo Transfer Newsletter (IETS), Vol28, No4: 14-26 (2014).

985,983 bovine embryos were transferred throughout in the world on 2013, and 411,198 (41.7%) in-vitro fertilized embryos were transferred.

2013年全世界共有985983個牛胚被移置，其中有411198 (41.7%) 個是體外受精胚

## IVM-IVF-IVC method

牛胚體外生產步驟



## Comparison of embryo production between MOET and OPU-IVF

### OPU體外受精胚與體內胚之比較

Embryo production System 胚生產系統	No. of OPU/MOET sessions 處理組數量	No. of follicles/CL 濾泡數量	No. of collected oocytes/ova 收集卵母細胞數量	No. of produced blastocysts 產製囊胚數量
OPU-IVF	60	43.4 ± 16.4	36.7 ± 18.3	11.8 ± 7.6 <sup>a</sup>
MOET	36	14.8 ± 9.8	9.3 ± 8.5	6.4 ± 6.3 <sup>b</sup>

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

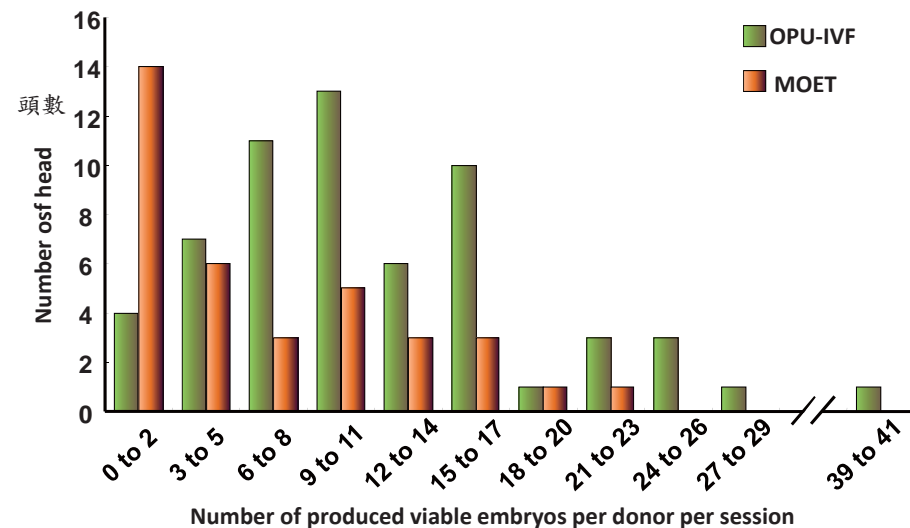
Data from more than 10 days OPU interval were collected.

具有不同上標同一列內的值(平均值±SD)的差異(P<0.01)收集的10多天時間OPU數據

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

## Distribution of embryo production between MOET and OPU-IVF

### MOET和OPU-IVF胚的生產比較



處理每頭供胚牛生產的可移置胚數量

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

## Embryo production by OPU-IVF in various donors

### 不同狀況的母牛進行OPU-體外受精生產的胚數量

Donors 供胚牛	No. OPU sessions (intervals) 處理組數量	No. follicles 濾泡數量	No. oocytes collected 收集卵母細胞數量	No. blastocysts produced 產製囊胚數量	% blastocyst 產製囊胚數量%
Dry cows	60 (>10)	43.4	36.7	11.8	41.6
Pregnant cows 懷孕母牛	16 (7)	43.1	39.3	12.3	40.7
Reproductive disorder 繁殖障礙	17 (>10)	36.2	30.0	7.8	29.3
9 months Calves 9月齡	28 (7)	29.8	22.4	5.0	26.0
6 months Calves 6月齡	2 (7)	45.0	32.5	5.0	19.5
FGT treatment 濾泡刺激處理組	8 (11)	29.3	19.0	12.8	68.1
SOV treatment 超級排卵處理組	8 (>10)	45.6	25.5	13.9	57.2

## Summary: embryo production by OPU-IVF

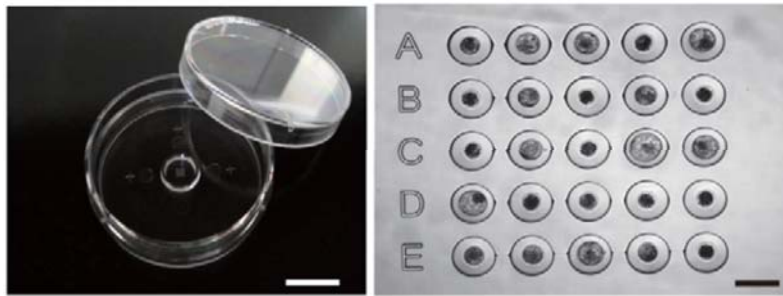
結論：OPU-IVF 牛胚生產

The efficiency of embryo production by OPU-IVF OPU-IVF 牛胚生產效能

- **OPU-IVF is more efficient embryo production system than MOET.** OPU-IVF比MOET效能高
- **There is no difference in the reproductive status, if ovaries have enough number of follicles.** 如果卵巢有濾泡數量不足的狀況，則在生殖效能方面沒有差別。
- **Small follicle < large follicle =/< in vivo matured** 小濾泡<大濾泡=/**體外成熟**
- **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.**  
利用OPU所獲得體內成熟卵母細胞在良好的供胚牛處理狀況下，每頭母牛能夠通過體外受精產生超過13囊胚。
- **However, there are some problems for embryo quality of blastocysts derived from x-sorted semen.** 利用選性的X精子受精的囊胚品質有缺陷

# Individual culture in IVF embryo

體外受精胚個別培養系統



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°)  
35毫米培養皿: 7毫米直徑在盤的中心25微孔 (280毫米直徑和深度160毫米, 錐度=7°)

Culture: 125µl of medium, 25 embryos  
5%O<sub>2</sub>, 5%CO<sub>2</sub>, 90%N<sub>2</sub> and saturated humidity  
125毫升培養小滴中置入25胚胎 5%O<sub>2</sub>, 5%CO<sub>2</sub>, 90%N<sub>2</sub>, 飽和濕度  
Sugimura et al, Biol Reprod, 2010

# Monitoring of embryo development by time-lapse cinematography

利用影像記錄系統監測胚胎發育狀況



Real-time culture cell monitoring system (Astec, Fukuoka, Japan)即時細胞培養影像系統

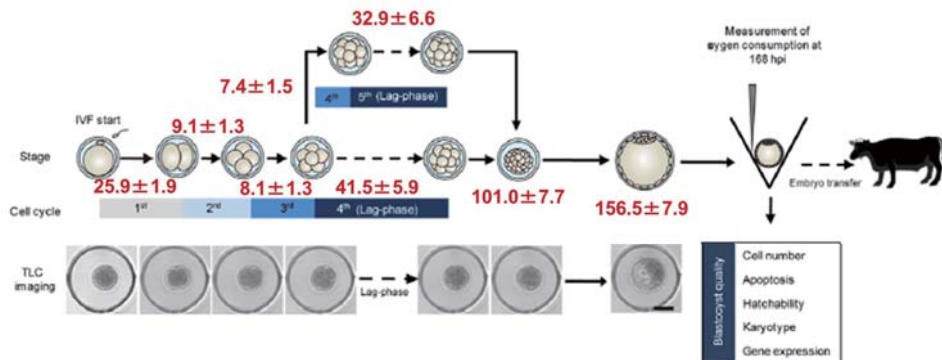
TLC: It take photos every 15min, then take 673 photos until finish the culture for 7 days  
每15分鐘拍照, 共拍673張照片, 直到完成培養7天

# Five prognostic factors for embryo quality

早期胚發育的5個觀察重點

- PF1: Timing at 1<sup>st</sup> cell cycle
- PF2: Number of blastomeres after 1<sup>st</sup> cell cycle
- PF3: Existence of fragments
- PF4: Number of blastomeres at lag-phase
- PF5: Oxygen consumption of blastocyst

PF1: 第一個細胞週期時間點  
PF2: 1細胞週期後卵裂細胞數  
PF3: 碎削片段的存  
PF4: 在胚發育阻滯期後卵裂細胞數  
PF5: 囊胚耗氧量



Sugimura et al, PLoS ONE (2012)

# Kinetics of embryo development affect the normality of chromosomes

胚胎發育的動力學是影響染色體是否正常的關鍵因素

Timing	End of first cleavage			Onset of lag-phase		
	Diploidi 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)	
		3-4		3/14 (21.4)	6-8	1/2 (50.0)
		3-4		0/9 (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)	
		3-4		0/9 (0)	6-8	3/9 (33.3)
		3-4		0/9 (0)	8-16	1/2 (50)

Sugimura et al, PLoS ONE, 2012

## Conception rates in IVF embryos selected by prognostic factors

早期胚發育觀察因素對體外胚移置後懷孕率之影響

Combinations	No. of transferred 胚移置數	No. of conceptions 懷孕數	% of conception 懷孕率%
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  $29.2 \pm 3.3$  kg that was close to that derived from AI embryos ( $28.7 \pm 4.2$  kg) and we observed no neonatal overgrowth or death.

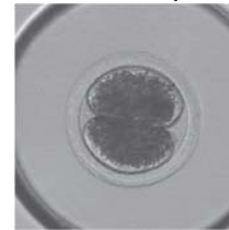
Sugimura et al, PLoS ONE (2012)

## Summary: Selection of embryos

結論：早期胚的品質篩選

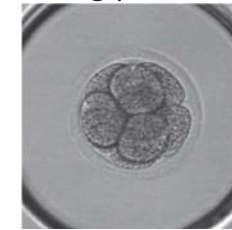
### Time-Lapse Cinematography

1st cell cycle



Less than 27 hpi  
Two blastomeres  
Without fragments  
受精後27小時分裂成  
完整無碎劑的2細胞

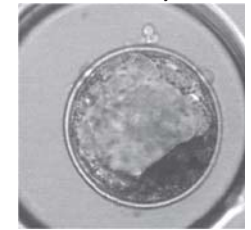
Lag-phase



Six blastomeres or more  
Without fragment  
以上且無碎劑

### Oxygen consumption

Blastocyst



High oxygen consumption  
高氧代謝量

IVF embryos: high competence of conception and delivery

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

胚發育影像紀錄系統對胚品質分析來說是一項很好的工具

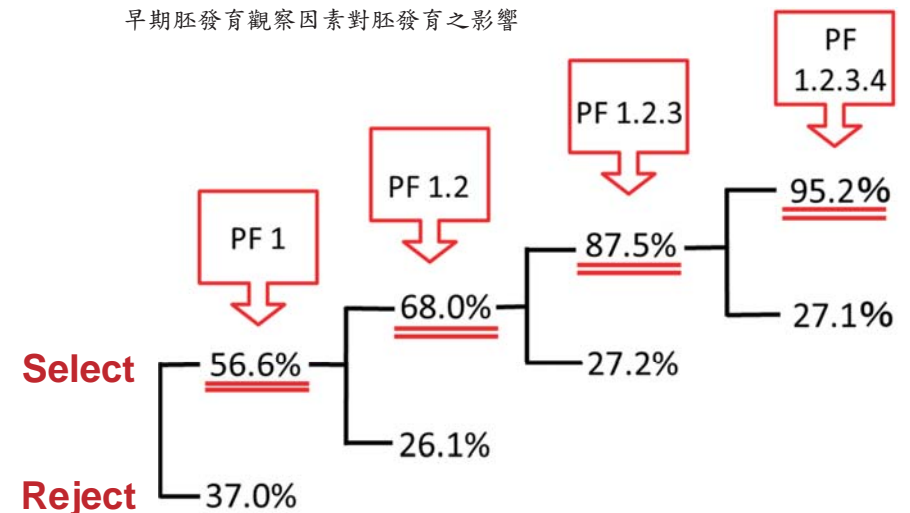
## Embryo selection system with four factors and three observations

To select healthy embryos, a combination of four factors were used as follows: 選擇健康的胚胎，四個因素的組合被使用如下：

- ① Time at which first cleavage occurred (less than 27 hpi, or less than 31 hpi, if in case of any zygotes did not cleaved at 27 hpi in the each culture dish) 第一次卵列發生 (小於27 HPI, 或小於31 HPI,)
- ② Two blastomeres after first cleavage at 31 hpi 受精後31小時有完整平均的2細胞
- ③ Absence of fragments after first cleavage at 31 hpi 無碎劑
- ④ Eight or more blastomeres at 55 hpi 受精後55小時有6個以上細胞

## Effect of prognostic factors in embryo development

早期胚發育觀察因素對胚發育之影響



Takayama et al., IETS meeting, 2015



# Pregnancy rates of selection system

胚篩選對懷孕率的影響

Morphological 形態鑑別	No. transfer 胚移植數	No. pregnant 懷孕數	Proportions 比例 (%)
Fair	21	12	57.1
good	24	13	54.2

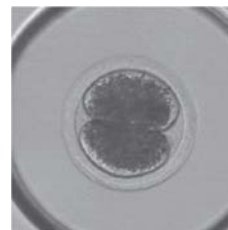
PF	No. transfer 胚移植數	No. pregnant 懷孕數	Proportions 比例 (%)
Selected	23	17	73.9 <sup>a</sup>
Rejected	22	8	36.4 <sup>b</sup>
Total	45	25	55.6

Takayama et al., 未發表

# In case of no TLC and oxygen consumption

沒有TLC並且有高氧氣代謝量狀況的早期胚

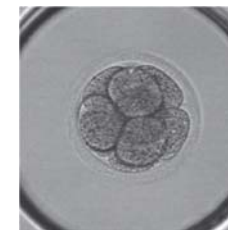
1st cell cycle



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**

可能獲得超過70%的懷孕率

# 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 factor can be useful for analyze sperm and oocyte quality



1. 利用OPU-IVF生產體外胚高效且穩定
2. 單獨胚體外培養和TLC
3. 胚選擇提高懷孕率
4. 早期胚發育觀察因素可用於分析精子和卵子的品質

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

提高胚胎品質能有效提升仔牛生產效能與受胚母牛利用效率