南部地區
女性的青春維護卵巢保養

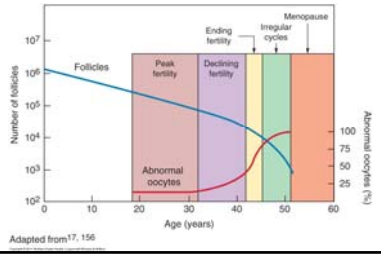
冷凍卵子與生育保存

成大醫院婦產部
暨生殖內分泌科
吳孟興 主任

20141207

生育年齡

- 女性相較於男性
  - – 生育期間較短 – 有年齡的限制
  - – 34歲後生育能力下降



Reproductive BioMedicine Online 2011 23, 334-340

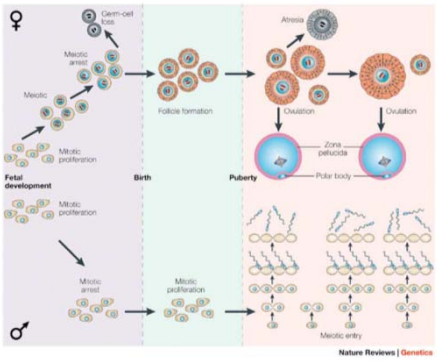
英國高齡婦女的活產率

Oocyte cryopreservation for elective deferral of reproduction

Table 1 UK live-birth rates for cycles completed in 2006.

Woman's age (years)	*Total no. of cycles*	*Live-birth rate (%)*
38	2968	17.9
39	2886	15.7
40	2219	12.5
41	1670	9.5
42	1110	6.6
43	706	3.5
44	375	2.4
45	184	2.7

Source: Human Fertilisation and Embryology Authority, http://www.hfea.gov.uk.



Reproductive BioMedicine Online 2011 23, 334-340

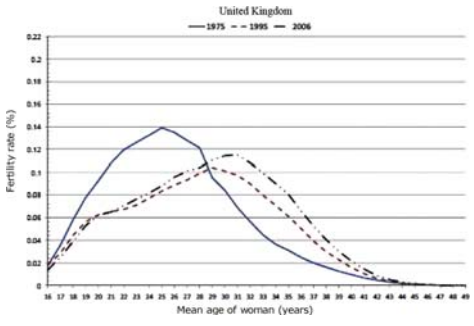
英國平均結婚和離婚的年齡

Table 2 Average age at marriage and divorce in England and Wales.

Year	*Age at first marriage (years)*		*Age at divorce (years)*	
	Males	*Females*	*Males*	*Females*
1961	25.6	23.1	–	–
1971	24.6	22.6	39.4	36.8
1981	25.4	23.1	37.7	35.2
1991	27.5	25.5	38.6	36.0
2000	30.5	28.2	41.3	38.8

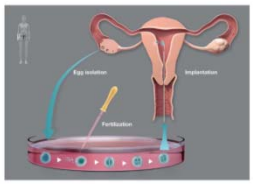
Reproductive BioMedicine Online 2011 23, 334-340

生育率與年齡有關



The Nobel Prize in Physiology or Medicine 2010
Robert G. Edwards

愛德華茲早在1950年代就發現體外受精可能是治療不孕的關鍵，直到1969年才首度在試管中培養出第一個人類體外受精卵，但植回母體卻無法分裂發育。 後來與婦科醫師Patrick Steptoe合作，以內視鏡技術從女性卵巢取出卵子，與男性精子配對，終於培育出能正常發育的受精卵，1978年7月25日，試管嬰兒露易絲布朗足月誕生，不孕治療也進入新的年代。



延伸生殖機率的年限
expand reproductive lifespan

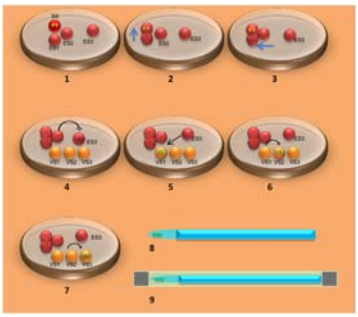
- 胚胎或卵子冷凍保存
- 醫療或非醫療因素

生育率保險

- 卵子冷凍技術的進步
  - 解凍後大於 90%的存活率與75%的受精率
- 雖然胚胎冷凍是標準冷凍保存的技術，卵子冷凍的好處是避免沒有精子的狀況
  - 例如單身女性或青少女
- 健康女性 (non-medical)– 生育年齡長與高教育程度

玻璃化冷凍

J Ovarian Res. 2013; 6: 15.



Indications of oocyte cryopreservation

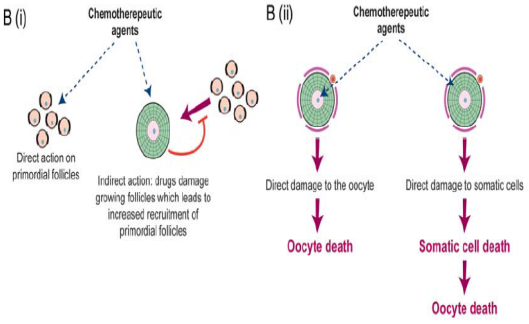
A. Nondonor oocyte cryopreservation	
Medical indications	Cancer patients receiving gonadotoxic therapies or undergoing surgery
	Noncancer patients receiving gonadotoxic therapies for various reasons
	Sickle-cell anemia
	Rheumatologic diseases
	Myelodysplastic syndrome
	Patients at risk of losing their fertility potential because of genetic abnormalities
	BRCA mutation carriers
	Turner syndrome
	Fragile X premutation
	Deletions of the X chromosome
	Twins of patients with premature ovarian failure
IVF related	Oocyte cryopreservation for those unable to cryopreserve embryos
	Poor responder patients
	Ovarian hyperstimulation syndrome (OHSS)
	Failure to obtain sperm for IVF
Elective oocyte cryopreservation	Deferring child bearing
B. Donor oocyte cryopreservation	

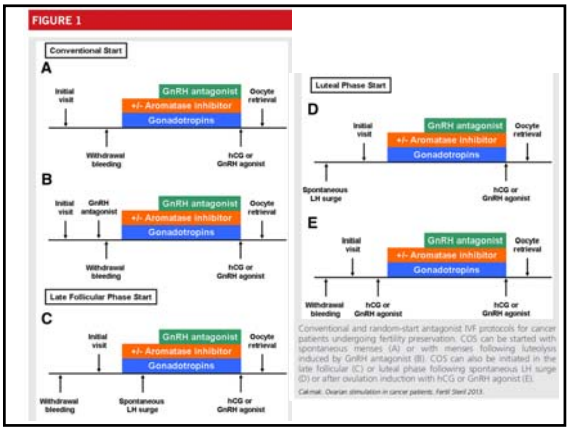
Curr Opin Obstet Gynecol. 2013 June ; 25(3): . doi:10.1097

卵子冷凍醫療性因素

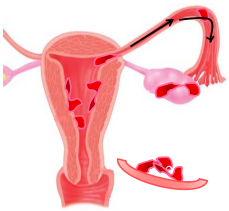
- **罹患癌症治療**
- **接受手術治療**

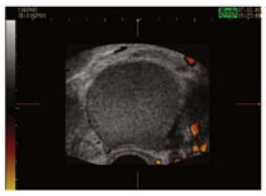
婦女罹患癌症化學治療
How chemotherapy toxic to ovary?





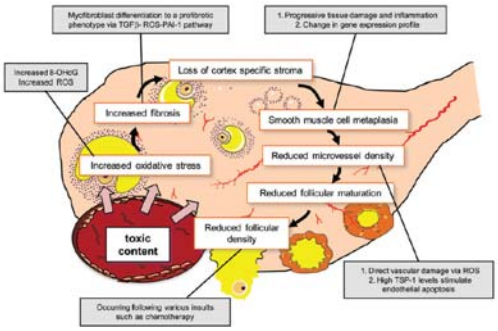
子宮內膜異位症
(Endometriosis)



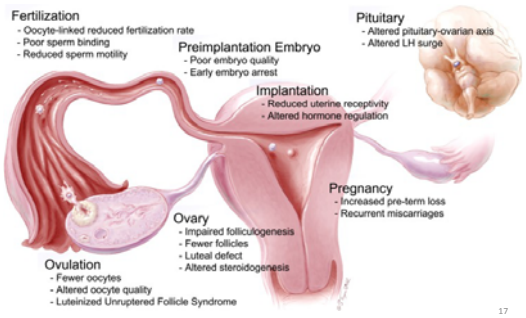


常見於約10%生殖年齡的婦女
並非單一的假設即可解釋

Hum Reprod Update, 2014;20(2) :217–230



Factors associated with reduced fecundity in women with endometriosis
Cell Tissue Res. 2012 September; 349(3): 849–862.



子宮內膜異位症手術的適應症

Table 5 Endometrioma: clinical variables to be considered when deciding whether to perform surgery or not in women selected for IVF [60]

Characteristics	Favours surgery	Favours expectant management
Previous interventions for endometriosis	None	≥1
Ovarian reserve [a]	Intact	Damaged
Pain symptoms	Present	Absent
Bilaterality	Monolateral disease	Bilateral disease
Sonographic feature of malignancy [b]	Present	Absent
Growth	Rapid growth	Stable

[a] Ovarian reserve is estimated based on serum markers or previous hyperstimulation cycles
[b] Sonographic feature of malignancy refers to solid components, locularity, echogenicity, regularity of shape, wall, septa, location and presence of peritoneal fluid

J Assist Reprod Genet (2010) 27:441–447

子宮內膜異位症手術治療

- 手術可以增加自然懷孕的機會，同時檢體的病理報告可以排除卵巢癌的危險
- 會盡量保留正常卵巢組織
- 仍然會造成卵巢的傷害、導致卵子存量下降以及藥物刺激產生卵子的反應降低，甚至產生早發性停經現象
- 手術後也不會使卵子品質改善

卵子存量指標

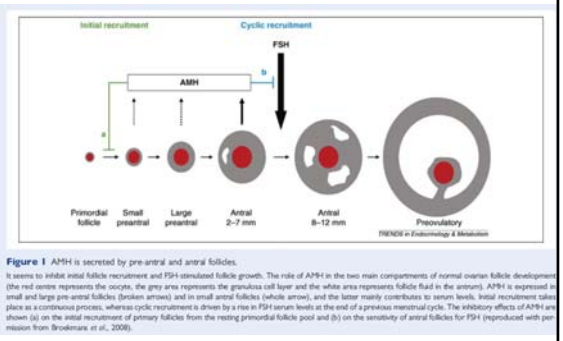


Figure 1 AMH is secreted by pre-antral and antral follicles.

It seems to inhibit initial follicle recruitment and FSH-stimulated follicle growth. The role of AMH in the two main compartments of normal ovarian follicle development (the red centre represents the oocyte, the grey area represents the granulosa cell layer and the white area represents follicle fluid in the antrum). AMH is expressed in small and large pre-antral follicles (broken arrows) and in small antral follicles (whole arrow), and the latter mainly contributes to serum levels. Initial recruitment takes place as a continuous process, whereas cyclic recruitment is driven by a rise in FSH serum levels at the end of a previous menstrual cycle. The inhibitory effects of AMH are shown (a) on the initial recruitment of primary follicles from the resting primordial follicle pool and (b) on the sensitivity of antral follicles for FSH (reproduced with permission from Broekmans et al., 2008).

Human Reproduction Update, Vol.16, No.2 pp. 113–130, 2010

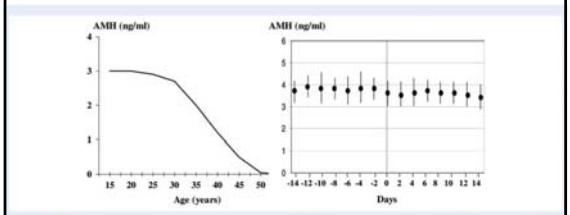


Figure 2 *Left*: Mean serum AMH levels show a reduction throughout reproductive life. Undetectable AMH levels after spontaneous menopause have been reported (constructed graphic). *Right*: Circulatory pattern of AMH during the menstrual cycle of young healthy women aged 18–24 years. Serum AMH levels have been shown to be stable throughout the menstrual cycle. Day 0 = day of LH surge (reproduced with permission from La Marca et al., 2006a).

Human Reproduction Update, Vol.16, No.2 pp. 113–130, 2010

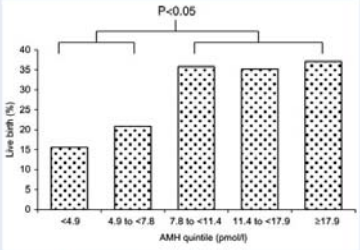


Figure 4 The mean live birth rate following IVF according to basal serum AMH levels.

In this prospective study of 340 patients it was demonstrated that the live birth rate dramatically increased with increasing basal AMH value. However this was valid only for women with basal levels <7.8 pmol/l. Above this value there was no discrimination for the live birth (reproduced with permission from Nelson et al., 2007).

Human Reproduction Update, Vol.16, No.2 pp. 113–130, 2010

Table V Comparison of characteristics of the most widely used markers of ovarian reserve

Characteristics for a good marker	Age	AMH	FSH	AFC
Prediction of poor response	+	+++	++	+++
Prediction of hyper response	+	+++	−	++
Low inter-cycle variability	+++	++	−	++
Low intra-cycle variability	+++	++	−	++
Blinded to the operator	+++	+++	+++	−
Applicable to all patients (a)	+++	+++	+	+
Cheapness	+++	−	−	−

(a) FSH and antral follicle count (AFC) are not informative in patients on hormonal contraception or GnRH agonist treatment. Moreover the count of antral follicles may be difficult in women with ovarian cysts or with previous pelvic surgery.

Human Reproduction Update, Vol.16, No.2 pp. 113–130, 2010

子宮內膜異位症的存在會造成血液中 AMH 值明顯下降

Table I Ovarian reserve in women with or without endometrioma.

	Endometrioma ($n = 30$)	Control ($n = 30$)	*P* value
Age (in years)	29.0 (5.4)	30.1 (4.4)	0.39
Nulligravid (%)	15 (50)	10 (33.3)	0.19
AMH (in ng/ml)	2.81 (2.15)	4.20 (2.26)	0.02
Total AFC	9.73 (4.77)	14.7 (4.11)	<0.01

Values are the mean (SD). AMH, anti-Müllerian hormone; AFC, antral follicle count.

Hum Reprod. 2013 ;28(8):2140-5.

因子宮內膜異位症手術會造成血液中
AMH 值明顯下降

Table II Markers of ovarian reserve in women before and after endometrioma excision.

	Preoperative ($n = 30$)	1 month after surgery ($n = 30$)	6 months after surgery ($n = 26$)
AMH (in ng/ml)	2.81 (2.15)	2.07 (1.47)$^{0.18}$	1.82(1.29)$^{0.02}$
Total AFC	9.73 (4.77)	11.0 (5.37)$^{0.28}$	10.4 (4.16)$^{0.63}$

Values are mean (SD). Superscripts are *P* values compared with preoperative values.

Hum Reprod. 2013 ;28(8):2140-5

卵子冷凍保存
Oocyte Cryopreservation

- 卵子冷凍保存可以減少若干的顧慮
  - 義大利或德國禁止胚胎的冷凍保存
- 卵子冷凍保存已經被廣泛的應用
  - 不僅在癌症患者延長生育的機會也提供健康女性調控生育或組織家庭的年限

生育年齡婦女考慮非醫療因素卵子冷凍的比率
Human Reproduction, Vol.26, No.3 pp. 655–661, 2011

Table I Intentions to freeze oocytes among women aged 21–40 years.

Would you consider to freezing oocytes for social reasons?	*n*	%	Group	%
Yes	32	3.1	Potential freezers	31.5
Maybe	291	28.4		
I don't know	171	16.7	Doubtful group	16.7
No	530	51.8	Non-freezers	51.8

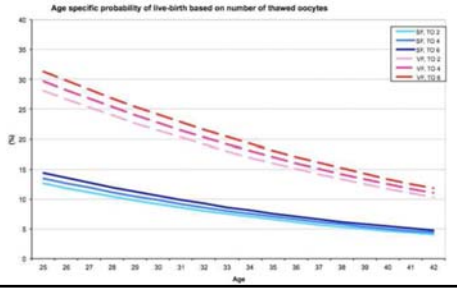
卵子冷凍技術的進步

- 分為三個因素:
  - 使用 intracytoplasmic sperm injection (ICSI)技術
  - 冷凍保護劑 cryoprotectants 的改良
  - 引用玻璃化冷凍vitrification技術

冷凍技術

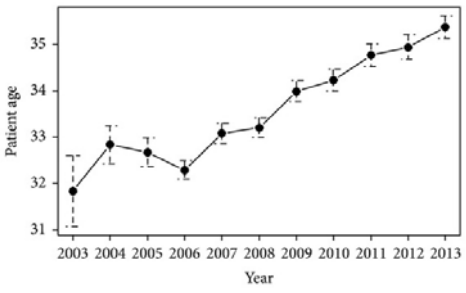
- 慢速冷凍
  - 較耗時且需要較多的液態氮氣
- 快速冷凍 – 玻璃化冷凍
  - 耗時較短且操作簡單

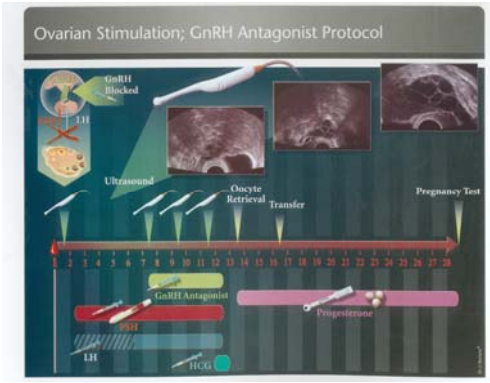
冷凍卵子解凍後的活產率
與年齡與冷凍方法有關
SF: slow freezing, VF: vitrification, TO: thawed oocytes, IO: injected oocytes, ET: embryos transferred
Fertil Steril. Aug 2013; 100(2): 492–9.e3.



近十年來接受冷凍技術的平均年齡持續增加

Biomed Res Int. 2014; 2014: 307268.





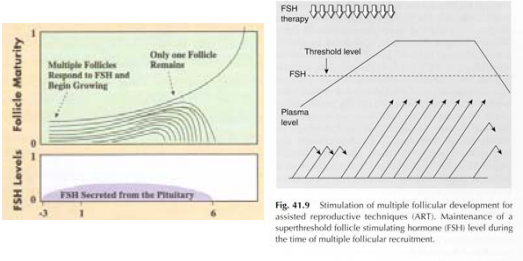
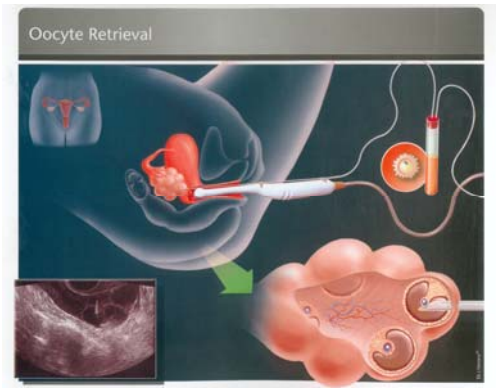
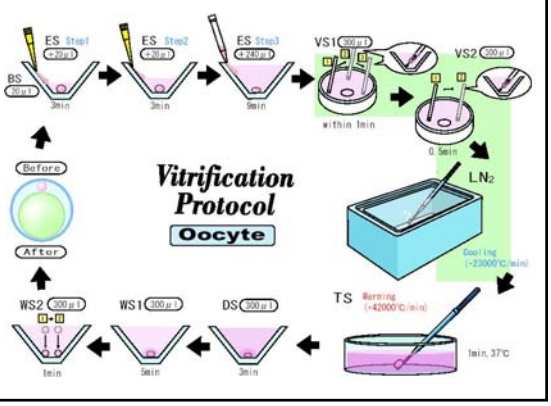
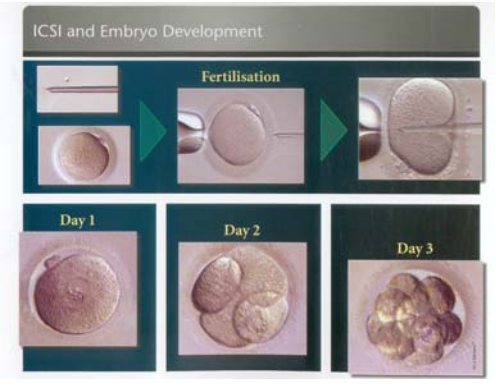
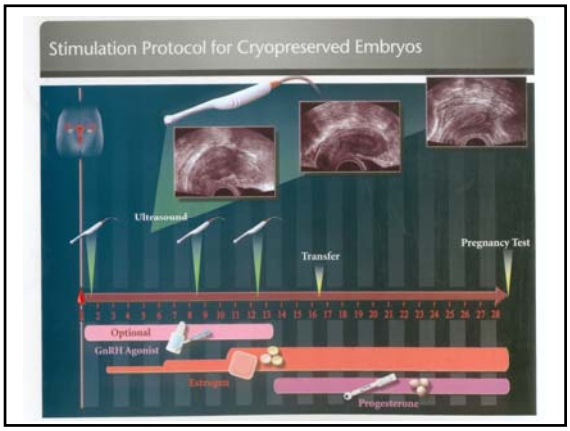


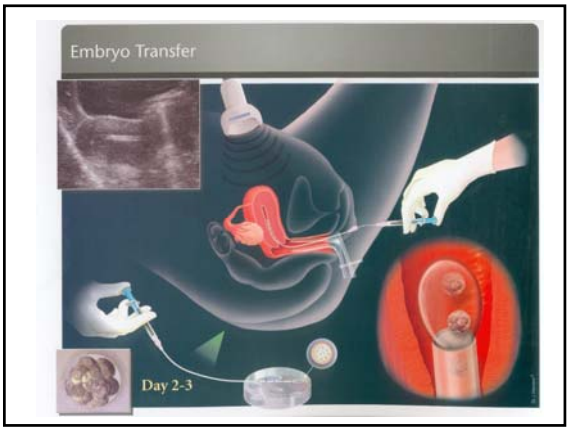
Fig. 41.9 Stimulation of multiple follicular development for assisted reproductive techniques (ART). Maintenance of a superthreshold follicle stimulating hormone (FSH) level during the time of multiple follicular recruitment.











人類卵子玻璃化冷凍後解凍生育機會
Outcomes of human oocyte vitrification

	Survival rate	Fertilisation rate	Implantation rate	Clinical pregnancy rate
Song et al. [2]	88 %	75 %	37 %	50 %
Cobo et al. [3]	92.5 %	58 %	39.9 %	50.2 %
Kim et al. [4]	81.0 %	73 %		80 %
Nagy et al. [5]	89.1 %	87 %	55.3 %	
Rienzi et al. [6]	84.7 %	75.2 %		34.2 %

J Assist Reprod Genet (2013) 30:203–206

結論

- 自1986年第一位冷凍卵子成功活產後，經過20年才因玻璃化冷凍技術成熟成功率才有明顯改善，而且與新鮮卵子成功率相當
- 臨床上應用於單身婦女或配合試管嬰兒技術使用
- 隨著社會性(social)冷凍卵子的個案增加，接受取卵手術時的年齡是往後生殖延續成功最重要的因素