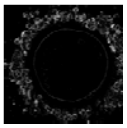
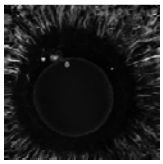
7th Workshop on Mammalian Folliculogenesis and Oogenesis
ESHRE Campus symposium Stresa, Italy - 19, 20 & 21 April 2012

Organised by the ESHRE Special Interest Group
Embryology, Reproductive Endocrinology & Task Force Basic Scientists in Reproductive Medicine



"Achievements and challenges of oocyte in vitro maturation in the human"



Giovanni Coticchio

Centro di Medicina della Riproduzione
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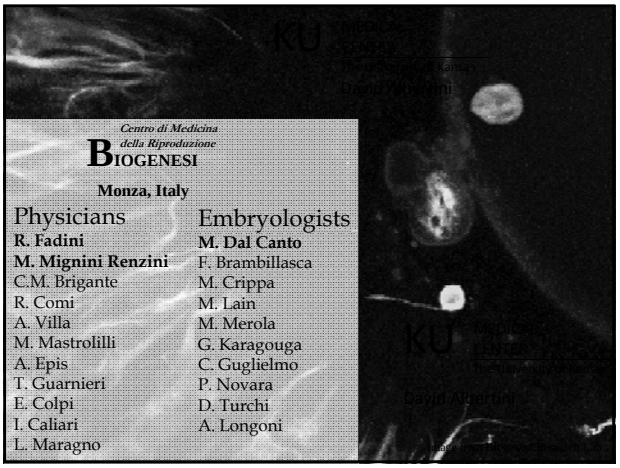
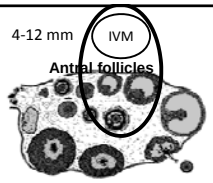


Human IVM: outline

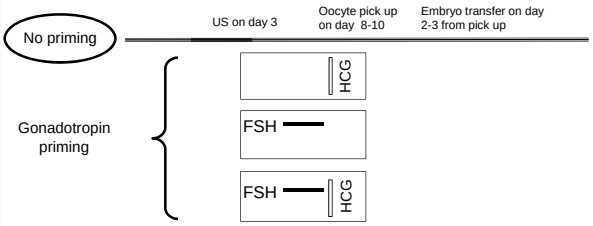
- Priming strategies
- Developmental potential of IVM oocytes
- Range of clinical applications
- The neglected organ – the endometrium
- IVM babies
- Culture media
- Critical changes occurring during IVM
- Potential new players for improved IVM systems

Early history of human IVM

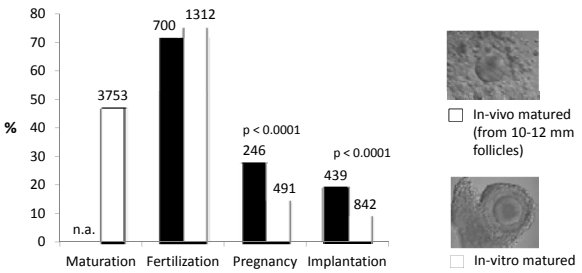
- **1965 Growing interest in oocyte IVM (Edwards 1965)**
- **1969 IVF of in vitro matured oocytes (Edwards 1969)**
- **1983 First delivery from IVM oocytes (GV from COS) (Veeck 1983)**
- **1991 Delivery after IVM in egg donation programme (Cha 1991)**
- **1994 Delivery from IVM oocytes of PCO patients (Trounson 1994)**
- **1995 Delivery after IVM and ICSI (Barnes 1995)**
- **1999 Delivery from IVM oocytes of normovulatory women (Mikkelsen 99)**



The (confounding) IVM priming strategies



Potential of in-vivo and in-vitro matured oocytes from IVM cycles primed with HCG



In-vivo matured (from 10-12 mm follicles)

In-vitro matured

Dal Canto el at, JARG, in press

IVM: for which patients?

Normovulatory?

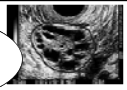
PCO/PCOS/OHSS

Male factor?

Cancer/fertility preservation?

Younger/older?

PCO/PCOS/OHSS



Authors	Cycles/ patients	N° of oocytes collected and mean	N° of oocytes matured (%)	N° of mature oocytes fertilized (%)	N ET	Mean of embryos transferrd	N° of clinical pregnancy per cycle (%)	Imp rate %
Son 2002	419	6860 16.4±7.1	5021 73.2 %	3967 79 %	1816	4.3 ± 0.9	137 32.7 %	11.6
Lin 2003	68/60	1528 22.5±10.1	1134 74.2%	852 72.8%	68	3.8 ± 1.0	23 33.8 %	10.5
Chian 2003	254	3079 11.9±6.2	2462 78 %	1679 69.2 %	865	3.4 ± 0.9	61 24.0 %	11.1
Cha 2005	203/139	3148 15.5±8.2	-	-	929	5.1 ± 2.1	41 20.2 %	5.5
Le Du 2005	45/33	509 11.4 ± 6.9	321 63 %	225 70 %	103	2.5	9 20.0%	10.9

FSH + hCG priming no hormonal priming hCG priming

MONZA- Italy12-13 December 2008

1ST EUROPEAN CONGRESS ON IN VITRO MATURATION OF HUMAN OOCYTES IN ASSISTED REPRODUCTION

PCO/PCOS/OHSS

IVM in Europe in recent years

			No	CPR	IR
Barak	**POL**	**PCO**	118	**33.3%**	**12%**
Child	**UK**	**PCO**	63	**32.0%**	**13%**
Frydman	**FRA**	**PCO**	234	**27.7%**	**12.4%**
Martinez	**ESP**	**PCO**	54	**34.7%**	**16.2%**

RBM Online - *Vol 19 No 3. 2009 343-351 Reproductive BioMedicine Online* Fadini et al.

Effect of different gonadotrophin priming on IVM of oocytes from women with normal ovaries: a prospective randomized study

Normovulatory?

Parameter	No Gnts	HCG	FSH	HCG FSH
No. of oocyte collections	93	93	95	98
No. of retrieved oocytes	494	495	469	536
Mean retrieved oocytes ± SD	5.3 ± 4.2	5.3 ± 4.1	4.8 ± 3.4	5.4 ± 3.5
No. of discarded oocytes	17	25	8	11
No. of immature oocytes cultured	477	442	461	416
No. of in-vivo-matured MII (%)	0	28 (5.7)	0	109 (20.3)
No. of in-vitro-matured MII after 30 h (%)	231/477 (48.4)	256/442 (57.9)	234/461 (50.8)	322/416 (77.4)
Total MII oocytes available (%)	231/477 (48.4)	284/470 (60.4)	234/461 (50.8)	431/525 (82.1)
No. of oocytes fertilized (%)	142/183 (77.6)	138/193 (71.5)	135/185 (73.0)	178/244 (73.0)
No. of embryo transfers	72	66	75	87
No. of embryos transferred	130	125	132	171
Mean no. of embryos transferred ± SD	1.8 ± 0.7	1.9 ± 0.7	1.7 ± 0.7	1.9 ± 0.8

RBM Online - *Vol 19 No 3. 2009 343-351 Reproductive BioMedicine Online* Fadini et al.

Effect of different gonadotrophin priming on IVM of oocytes from women with normal ovaries: a prospective randomized study

Normovulatory?

Outcome	No Gnts	HCG	FSH	HCG FSH
CPR-ITT	11/100 (11.0)	5/100 (5.0)	13/100 (13.0)	26/100 (26.0)
CPR-OC	11/93 (11.8)	5/93 (5.4)	13/95 (13.7)	26/98 (26.5)
CPR	11/72 (15.3)	5/66 (7.6)	13/75 (17.3)	26/87 (29.9)
Implantation rate	12/130 (9.2)	5/125 (4.0)	14/132 (10.6)	28/171 (16.4)
Miscarriage rate	2/11 (18.2)	0/5 (0.0)	3/13 (23.1)	4/26 (15.4)

RBM Online - *Vol 18 No 2. 2009 251-261 Reproductive BioMedicine Online* Fadini et al.

Predictive factors in in-vitro maturation in unstimulated women with normal ovaries

Age	≤ 36 years
Basal FSH	< 10 mUI/ ml
Basal 17 ß Oestradiol	≤ 250 pmol/L
Antral Follicle Count	> 5

Normovulatory?

J Assist Reprod Genet. 2011 Jun. 15;28(6):501–508. Fadini et al.

Anti-mullerian hormone as a predictive marker for the selection of women for oocyte in vitro maturation treatment

	Univariate linear regression analysis			Multivariate linear regression model		
	Coef.	95% IC	p-value	Coef.	95% IC	p-value
Age	-0.17	-0.33–-0.01	0.038	-0.11	-0.28–0.06	0.196
FSH	-0.36	-0.63–-0.10	0.008	-0.06	-0.34–0.22	0.690
17-β oestradiol	-0.03	-0.07–-0.00	0.044	-0.01	-0.04–0.02	0.602
AFC	0.36	0.23–0.49	< 0.0001	0.13	-0.02–0.28	0.092
AMH	0.66	0.48–0.84	< 0.0001	0.54	0.30–0.77	< 0.0001

An AMH value of 1.28 ng/ml was identified as a threshold for the prediction of the retrieval of at least 5 oocytes

Outcome of cycles of oocyte in vitro maturation requiring testicular sperm extraction for nonobstructive azoospermia

Fadini et al., 2011 Fertility and Sterility® Vol. 96, No. 2, August 2011

Male factor?

Laboratory data of 27 cycles in which 21 women were treated with IVM and male partners were affected by NOA and underwent TESE.

No. of oocyte collections	27
No. of retrieved oocytes	138
Mean (+SD) no. of retrieved oocytes	5.1 + 2.6 (range 1–15)
No. of mature oocytes at retrieval	25
No. of cultured immature oocytes	112
No. of in vitro matured oocytes (%)	53 (47.3)
No. of discarded oocytes	
Total no. of mature oocytes av	
No. of inseminated oocytes	
No. of fertilized eggs (%)	
No. of embryo transfers	
No. of embryos transferred	
Mean no. of transferred embryo	

Clinical outcome of 27 IVM-TESE cycles.

β-hCG positive	8
Clinical pregnancies	6
Pregnancy rate per cycle	22.2%
Pregnancy rate per embryo transfer	27.3%
Implantations	6 (16.7%)
Abortions	1
Live births	5

Cancer/fertility preservation?

Retrieval of immature oocytes from unstimulated ovaries followed by in vitro maturation and vitrification: A novel strategy of fertility preservation for breast cancer patients

Huang et al.

The American Journal of Surgery (2010) 200, 177–183

Abstract

BACKGROUND: We report a novel fertility preservation strategy that may be useful for young breast cancer patients who present with time constraints or concerns about the effect of ovarian stimulation.

METHODS: The protocol involves retrieval of immature oocyte from unstimulated ovaries followed by in vitro maturation (IVM), and vitrification of oocytes or embryos.

RESULTS: Thirty-eight patients (age 24–45 years) underwent vitrification of oocytes (n = 18) or embryos (n = 20). The mean ages were 33.1 ± 5.0 years and 34.7 ± 4.8 years, respectively. The mean days required to complete the egg collection was 13 days. The median numbers of vitrified oocytes and embryos per retrieval were 7 (range 1–22) and 4 (range 1–13), respectively.

CONCLUSIONS: The strategy of immature oocyte retrieval without ovarian stimulation followed by IVM and oocyte or embryo vitrification, which does not increase the serum estradiol level and delay cancer treatment, represents an attractive option of fertility preservation for many breast cancer patients.

Cancer/fertility preservation?

IVM cycles performed at Biogenesi for fertility preservation purposes

No. of women	38
Mean age±SD (range)	35.7±4.1 (26-40)
IVM cycles	43

Cancer/fertility preservation?

IVM cycles performed at Biogenesi for fertility preservation purposes

No. of pts	No. of cycles	Tumor type
32	37	Breast
2	2	Ovarian
1	1	Colon
1	1	Neuroendocrine
1	1	Lymphoma
1	1	Lung

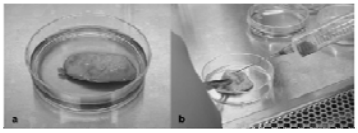
Cancer/fertility preservation?

Immature oocyte recovery, maturation, and cryopreservation in cancer patients

No. of retrieved oocytes	229
Mean ± SD of retrieved oocytes per cycle	5.7±5.3
No. of in vitro matured and vitrified oocytes (%)	156 (68.1)
Mean ± SD of vitrified oocytes per patients	3.6±4.8
No. of warming cycles	0

Cryopreservation of immature oocytes as an adjunct to ovarian cortex cryobanking

Cancer/fertility preservation?



Retrieval of immature oocytes from ovarian tissue followed by in vitro maturation and vitrification of mature oocytes.

Patient no.	Age	Cancer type	Day of menstrual cycle	Surgical procedure	No. of GV oocytes retrieved from ovarian tissue	No. of MII oocytes following IVM	Maturation rate (%)	No. of MII oocytes vitrified
1	21	Hodgkin lymphoma	2	Ovarian wedge resection	3	3	100	3
2	35	Breast	19	Oophorectomy	1	1	100	1
3	18	Hodgkin lymphoma	5	Ovarian wedge resection	4	2	50	2
4	30	Rectal cancer	14	Ovarian wedge resection and oophoropexy	3	2	67	2

Huang et al., 2010

Cryopreservation of immature oocytes as an adjunct to ovarian cortex cryobanking for cancer patients

Cancer/fertility preservation?

Patients	Age	Retrieved oocytes	Cryopreserved oocytes
	31	3	0
	20	0	0
	28	1	0
	14	12	4
5	23	0	0
6	32	2	0
7	19	0	0
8	35	0	0
9	26	4	0
10	15	6	5
11	36	0	0
12	31	0	0
13	36	4	0
14	32	0	0

Oocytes were retrieved from slices of ovarian cortex, not from whole ovaries

Biogenesi, unpublished data

CASE REPORT

Embryo transfer following in vitro maturation and cryopreservation of oocytes recovered from antral follicles during conservative surgery for ovarian cancer

Fadini et al., JARG, in press

a 38-year-old woman with a previous history of infertility requiring surgery for ovarian adenocarcinoma

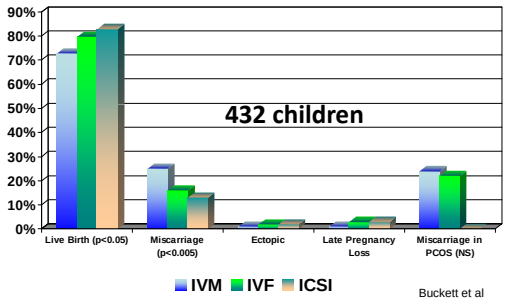
Conservative laparotomy, right annexectomy, and cyst excision from the left ovary. Contextual and opportunistic retrieval of immature oocytes from antral follicles of the left ovary.

- 3 COCs collected and matured in vitro
- 3 mature oocytes vitrifiied
- 2 oocytes survived after warming (15 months from surgery)
- 2 oocytes microinjected
- 1 fertilized egg
- 1 embryo at the 2-cell stage transferred on day 2
- No pregnancy, but proof of principle

IVM births

More than 2,500 IVM babies have been born so far, mostly unreported

Pregnancy outcome in IVM, IVF and ICSI cycles



Buckett et al
Obstet Gynecol 2007;110:885-91

Almost 200 babies born at Biogenesi

	Singletons IVM (=153)	Singletons ICSI (n=148)	Twins IVM (=21)	Twins ICSI (n=23)
Babies born	153	148	42	46
Mean maternal age at delivery (ys)	33,3 ± 3,2	34,9 ± 3	35,4 ± 3,2	35,6 ± 2,6
Mean birth weight (g)	3269 ± 616	3091 ± 668	2283 ± 555	2432 ± 540
Proportion LBW (<2,500 g)	6,5%	10,8%	61,9%	43,5%
Proportion VLBW (<1,500 g)	2,0%	2,7%	7,1%	6,5%
Proportion of macrosomic (> 4,200 g)	3,9%	3,4%	0,0%	0,0%
Males	48,4%	43,9%	57,1%	47,8%
Females	51,6%	56,1%	42,9%	52,2%
Mean gestational age (wk)	38,6 ± 2,3	38,7 ± 2,5	35,2 ± 2,7	37 ± 2,6
Proportion delivery less than 37 wk	17,0%	15,5%	71,4%	43,5%
Proportion delivery less than 32 wk	2,0%	2,7%	9,5%	4,3%
Apgar score at 1 minute	9,1 ± 1,1	9,1 ± 1,1	8,3 ± 2	9 ± 1,1
Apgar score at 5 minutes	9,9 ± 0,5	9,9 ± 0,5	9,6 ± 0,7	9,7 ± 0,7
Major congenital abnormalities	0	2	0	1
Minor congenital abnormalities	8	6	2	4

Unpublished data

The neglected organ: the endometrium

Clinical outcome of non–hCG-primed oocyte in vitro maturation treatment in patients with polycystic ovaries and polycystic ovary syndrome

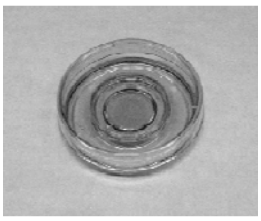
De Vos et al. Fertility and Sterility® Vol. 96, No. 4, October 2011

Comparative clinical outcomes of fresh and vitrified-warmed IVM embryo transfer.

	Fresh	Vitrified-warmed	*P* value
Clinical pregnancy rate	5/53 (9.4%)	7/22 (31.8%)	.033
Positive hCG	7/53 (13.2%)	9/22 (40.9%)	.008
Implantation rate	5/72 (6.9%)	7/32 (21.9%)	.043

"A non–hCG-primed IVM system in PCO or PCOS performs poorly when embryos are transferred in a fresh cycle. Transfer of vitrified-warmed IVM embryos in an artificial cycle leads to significantly improved clinical outcomes."

In vitro maturation systems



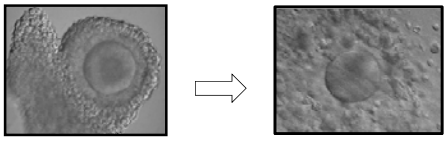
Have IVM systems been improved in the last 15-20 years?

Culture condition	Trounson et al., 1994	Fadini et al., 2011
Culture device	4-well plates	4-well plates
Culture medium	E-MEM (aspecific)	Origio (aspecific)
FSH	75 mIU (HMG)	75 mIU (rFSH)
HCG (LH-like)	500 mIU (HCG)	100 mIU (HCG)
Estradiol	1 mg/ml	-
Protein supplement	Maternal serum	HSA

Easy answer …. NO

What do we expect from novel IVM systems?

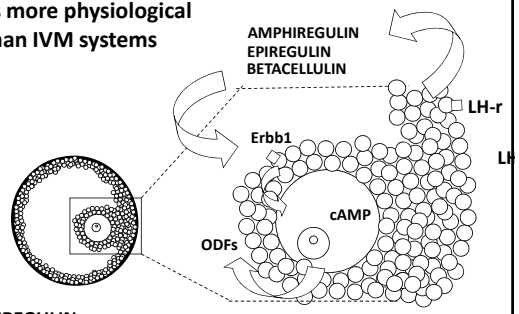
Imperative to improve oocyte quality **AND** quantity



GV stage MII stage

Current maturation rates are low, ranging a mere 50%

Towards more physiological human IVM systems



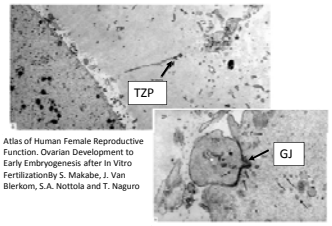
AMPHIREGULIN

- **Accumulates in preovulatory follicles after HCG administration**
- **Levels are associated with oocyte maturity at retrieval**

…. But

No evidence yet of a possible ability of amphiregulin to improve oocyte maturation rate and quality in vitro

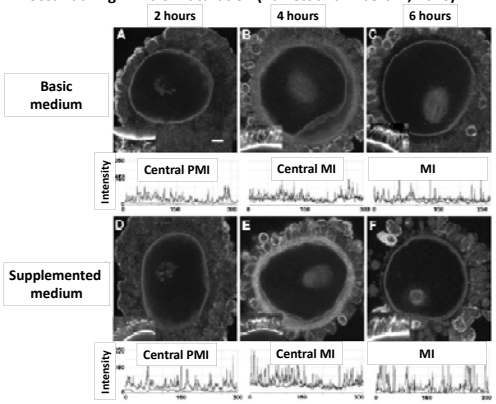
Direct CC-oocyte interaction during IVM



Atlas of Human Female Reproductive Function. Ovarian Development to Early Embryogenesis after In Vitro FertilizationBy S. Makabe, J. Van Blerkom, S.A. Nottola and T. Naguro

- Is it affected by in vitro conditions?
- …. And if yes, what are the consequences?

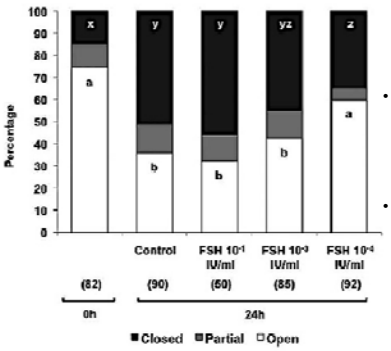
TZP retraction, GV centration and increase in spindle size occur during in vitro maturation (Barrett and Albertini, 2010)



BIOLOGY OF REPRODUCTION 85, 1252–1259 (2011)

Gap Junction-Mediated Communications Regulate Chromatin Remodeling During Bovine Oocyte Growth and Differentiation Through cAMP-Dependent Mechanism(s)

Alberto M. Luciano,[2] Federica Franciosi, Silvia C. Modina, and Valentina Lodde



- **Culture causes closure of CC-oocyte gap junctions in bovine oocytes. This affects chromatin condensation during meiosis and, ultimately, developmental ability**
- **Maintenance of gap junction perviety is sensitive to FSH concentration**

CC-oocyte communication in the human

Human COC from unstimulated patient

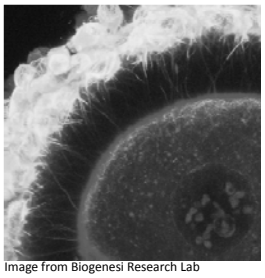


Image from Biogenesi Research Lab

During IVM

- Are TZPs retracted?
- Are gap junctions left open?
- How can we preserve functional communication ?

Conclusion

- In the human, in vitro matured oocytes are developmentally inferior in comparison to their in vivo counterparts
- This has not prevented IVM to become an increasingly useful strategy for a range of clinical conditions. The field of application of IVM, though, should be defined more precisely
- More than 2500 births have been obtained from IVM oocytes. Data on the health of babies are scant, but current information does not suggest an increase in congenital abnormalities
- Proper endometrial preparation could significantly improve success rates
- IVM systems are definitely inadequate and have not evolved significantly over almost two decades
- Identification of key players of the maturation game could lead to a major improvement of IVM systems and, ultimately, clinical efficiency