

IVM and vitrification Better option than before?

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Does IVM improve success rates of IVF in women with PCO/PCOS ?

- Baby take home rate
- Multiple pregnancy rate
- OHSS risk
- How are the offsprings doing ?
- Vitrification of human immature oocytes
- Spindel problems ?
- Survival rate
- Offspring and birth defects
- Safety
- New perspectives



Does IVM improve success rates of IVF in women with PCO/PCOS ?

Table II. Biological data and birth reports after *in vitro* maturation from unstimulated cycles in polycystic ovarian syndrome patients

| | Cycles | Immature oocytes | | Maturation | | Fertilization | | Transfers | | Pregnancies | | Births |
|------------------------------------|--------|------------------|------|------------|------|---------------|------|-----------|---------|-------------|---------|---------|
| | | Total | Mean | Total | % | Total | % | n | Embryos | Biochemical | Ongoing | |
| Ersson <i>et al.</i> (1996) | 9 | 308 | 13.4 | 169 | 60.4 | 41 | 13 | 17 | 1 | | | 1 |
| Barnes <i>et al.</i> (1995) | 3 | | | | 27.6 | 62 | 2 | 4 | 1 | | | 1 |
| Barnes <i>et al.</i> (1996) | 9 | 165 | 16.5 | 102 | 60.0 | 27 | 26 | | | | | 0 |
| Cha and Cha (1998) | 3 | 832 | 499 | 60.0 | 364 | 73 | 64 | 306 | 16 | | | 16 |
| Chan <i>et al.</i> (1999a) | 3 | 17 | 13 | 76.5 | 10 | 77 | 4 | 10 | 2 | 2 | | 4 |
| Chan <i>et al.</i> (1999b) | 25 | 249 | 200 | 84.0 | | | | | | | | 5 |
| Chan <i>et al.</i> (2000) | 24 | 183 | 142 | 77.6 | 125 | 88 | 24 | 63 | 8 | 6 | | 6 |
| Cha <i>et al.</i> (2000) | 94 | 1139 | 13.6 | 708 | 62.0 | 481 | 75 | 85 | 416 | 23 | | 20 |
| Mikolajewicz and Lindenberg (2001) | 12 | 81 | 36 | 44 | 25 | 69 | 30 | 53 | 7 | | | 0 |
| Child <i>et al.</i> (2001) | 68 | | 11.3 | | | | 79 | 67 | 217 | 20 | | 10 |
| Chan <i>et al.</i> (2001) | 1 | 63 | | 22 | | 15 | | 1 | 3 | | | 2 |
| Abdel-hadi <i>et al.</i> (2001) | 1 | 12 | | 6 | 50 | 4 | 67 | 1 | 3 | | | 2 |
| Child <i>et al.</i> (2002) | 107 | 1102 | 10.3 | 834 | 75 | 652 | 76 | 107 | 3 | 28 | | 17 |
| Ngarkh <i>et al.</i> (2002) | 1 | 16 | | 11 | | 7 | 64 | 1 | 3 | 1 | | 1 |
| Seem <i>et al.</i> (2002) | 1 | 61 | | 40 | 65.6 | 38 | 95 | 1 | 3 | 2 | | 2 |
| Kyren <i>et al.</i> (2002) | 1 | 12 | | 12 | | 12 | | 1 | 3 | 2 | | 2 |
| Liu <i>et al.</i> (2003) | 33 | 362 | 23.1 | 548 | 71.9 | 383 | 69.5 | 33 | 125 | 12 | | 12 |
| Our study | 45 | 509 | 11.4 | 321 | 63.0 | 70 | 40 | 103 | 11 | 6 | | 5 (1+1) |



IVM/ICSI/IVF

- *Multiple pregnancy*
- Of the ART pregnancies 267 (78%) were singleton pregnancies (IVM 31; IVF 132; ICSI 104), 66 (19%) were twin pregnancies (IVM 9; IVF 35; ICSI 22), and 11 (3%) were triplet pregnancies (IVM 2; IVF 5; ICSI 4). Of all the non-ART pregnancies 13,182 (98%) were singleton pregnancies, 201 (1.5%) were twin pregnancies, and 4 (0.03%) were triplet pregnancies.

Buckett: Obstet Gynecol, Volume
110(4), October 2007.885-891



Does IVM improve success rates of IVF in women with PCO/PCOS ?

Table 1. Results of 107 Age- and Diagnosis-Matched IVM and IVF Treatment Cycles in Infertile Women With Polycystic Ovaries

| | IVM | IVF | OR (95% CI) |
|---|------------------|---------------------------|-------------------|
| No. of cycles | 107 | 107 | |
| Age (y) | 32.8 ± 4.2 | 33.1 ± 4.1 | |
| Total injected units (ampoules) of follicle-stimulating hormone | 0 | 2355 ± 833 (31.4 ± 11.1)* | |
| Oocytes collected | 10.3 ± 7.6 | 14.9 ± 6.5* | |
| Metaphase II stage oocytes | 7.8 ± 4.9 | 12.0 ± 5.4* | |
| Fertilized 2PN embryos | 6.1 ± 3.8 | 9.3 ± 4.4* | |
| Cleaving embryos | 5.8 ± 3.7 | 8.6 ± 4.2* | |
| Embryos transferred (range) | 3.2 ± 0.9 (1-5) | 2.7 ± 0.8 (1-6)* | |
| Embryos cryopreserved (range) | 0.8 ± 2.3 (0-14) | 1.2 ± 3.0 (0-16) | |
| Pregnant [n (%)] | 28 (26.2) | 41 (38.3) | 0.57 (0.31, 1.06) |
| Implantation rate (%) | 9.5 | 17.1* | 0.51 (0.31, 0.84) |
| Clinical pregnancy [n (%)] | 23 (21.5) | 36 (33.7) | 0.54 (0.28, 1.04) |
| Live birth [n (%)] | 17 (15.9) | 28 (26.2) | 0.53 (0.26, 1.10) |
| Multiple live births [n (% of total live births)] | 7 (41.2) | 10 (37.0) | 1.26 (0.30, 5.11) |
| Twins | 6 | 9 | |
| Triplets | 1 | 1 | |
| Moderate or severe ovarian hyperstimulation syndrome | 0 | 12 (11.2%)* | |

IVM = in vitro maturation; IVF = in vitro fertilization; OR = odds ratio; CI = confidence interval.

Results are means ± standard deviations unless stated.



Table 1. Results of 107 Age- and Diagnosis-Matched IVM and IVF Treatment Cycles in Infertile Women With Polycystic Ovaries
From: Child: Obstet Gynecol, Volume 100(4), October 2002.665-670

IVM/IVF/ICSI congenital abnormality

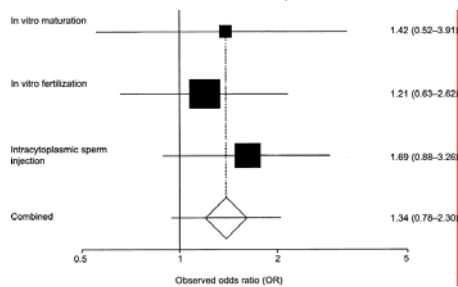
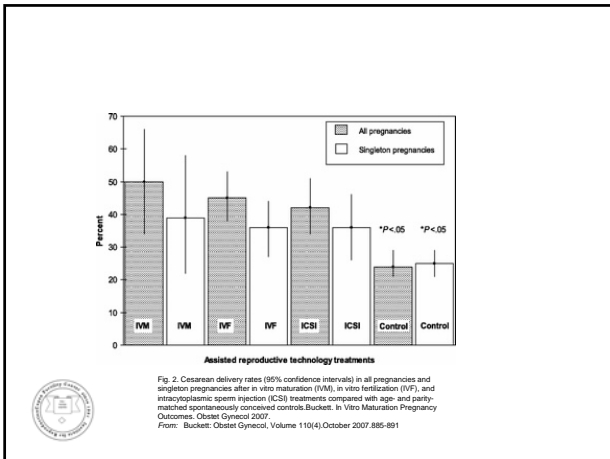


Fig 1. Observed odds ratio for any congenital abnormality after conception with in vitro maturation (IVM), in vitro fertilization (IVF), and intracytoplasmic sperm injection (ICSI) compared with age- and parity-matched spontaneously conceived controls. Buckett. In Vitro Maturation Pregnancy Outcomes. Obstet Gynecol 2007.
From: Buckett: Obstet Gynecol, Volume 110(4), October 2007.885-891





Does IVM improve success rates of IVF in women with PCO/PCOS ?

Table 2. Comparison of Outcomes in Singleton Pregnancies Conceived After In Vitro Maturation, In Vitro Fertilization, or Intracytoplasmic Sperm Injection With Spontaneously Age- and Parity-Matched Controls

| | IVM (n=31) | IVF (n=133) | ICSI (n=104) | Controls (n=338) | P (vs Controls) |
|--|------------|--------------|--------------|------------------|-----------------|
| Mean birth weight (g) | 3,482* | 3,209 | 3,163 | 3,260 | .048* |
| Proportion LBW (less than 2,500 g) | 1/31 (3) | 14/133 (10) | 15/104 (14) | 30/338 (9) | NS |
| Proportion VLBW (less than 1,500 g) | 0/31 (0) | 1/133 (1) | 3/104 (3) | 8/338 (2) | NS |
| Proportion of macrosomic infants (more than 4,200 g) | 3/31 (10) | 5/133 (4) | 2/104 (2) | 12/338 (4) | NS |
| Mean gestational age (wk + d) | 39 + 3 | 38 + 3* | 38 + 0* | 39 + 6 | <.001* |
| Proportion delivery less than 37 wk | 2/31 (6) | 23/133 (17)* | 25/104 (24)* | 18/338 (5) | <.01* |
| Proportion delivery less than 34 wk | 0/31 (0) | 5/133 (4) | 8/104 (8) | 4/338 (2) | NS |

IVM, in vitro maturation; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection; LBW, low birth weight; NS, not significant; VLBW, very low birth weight.
Data are expressed as n (%), except where otherwise indicated.
* Significantly different from control.

Table 2. Comparison of Outcomes in Singleton Pregnancies Conceived After In Vitro Maturation, In Vitro Fertilization, or Intracytoplasmic Sperm Injection With Spontaneously Age- and Parity-Matched Controls
From: Buckett. Obstet Gynecol, Volume 110(4), October 2007:885-891

Does IVM improve success rates of IVF in women with PCO/PCOS ?

- **CONCLUSION:** All ART pregnancies are associated with an increased risk of multiple pregnancy, cesarean delivery, and congenital abnormality. Compared with IVF and ICSI, IVM is not associated with any additional risk.
- **LEVEL OF EVIDENCE:** II

Buckett. In Vitro Maturation Pregnancy Outcomes. Obstet Gynecol 2007

Risk of OHSS

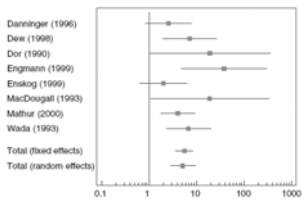
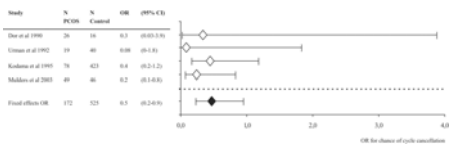


Fig. 3. Forest plot of cohort studies showing odds ratios with 95% confidence interval.



Odds ratio (OR) for cancellation rate comparing polycystic ovary syndrome (PCOS) patients and matched controls



Heijnen, E.M.E.W. et al. Hum Reprod Update 2006 12:13-21; doi:10.1093/humupd/dmi036

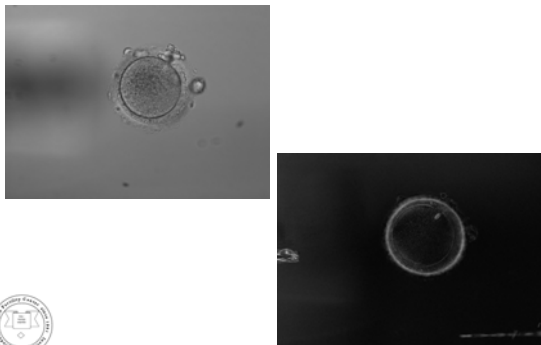
In our own study of 120 IVF cycles a 30% cancellation rate is recorded



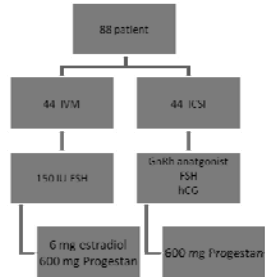
Human Reproduction Update

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Spindel apparatus in IVF



Material for spindle comparison between ICSI and IVM ova

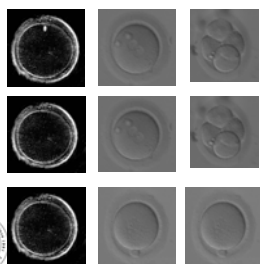


Does IVM improve success rates of IVF in women with PCO/PCOS ?

Are the oocytes after maturation healthy ?

| | Total no oocyte aspirated/mean per patient) | Total no of MFI/mean per patient) | Mean number of MFI oocytes with spindle present | Fraction of MFI oocytes with later PN formation (%) | Fraction of MFI later having normal PN formation, spindle present and cleaved | Fraction of oocytes with 0-45° angle for the spindle position |
|------------------|---|-----------------------------------|---|---|---|---|
| In vivo matured | 259 (5,9) | 224 (5,0) | 2,3 | 67 % | 34% | 81 % |
| In vitro matured | 215 (4,9) | 117 (2,7) | 1,5 | 53 % | 30% | 94 % |
| Significance | Not significant | P<0,05 | P<0,05 | Not significant | Not significant | Not significant |

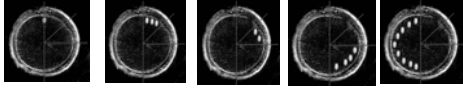




| | IVM | ICSI |
|-----------------|----------|-----------|
| MII (n) | 116 | 224 |
| MII + sp (n) | 65 (56%) | 100 (44%) |
| MII +sp +PN +CI | 29 (25%) | 69 (31%) |
| MII -sp +PN +CI | 20 (17%) | 49 (22%) |
| MII -sp -PN -CI | 24 (21%) | 44 (20%) |



Spindle position



| Angle | 0° | 1-45° | 46-90° | 91-180° | >181° |
|-------|-------|-------|--------|---------|-------|
| IVM | 36,9% | 56,9% | 4,6% | 0% | 1,5% |
| ICSI | 25,7% | 63,3% | 8,9% | 1,9% | 0% |



Effect of in vitro maturation on spindle position in human oocytes .
A prospective comparative study on patients with male factor.

Results: We found the same mean number of oocytes retrieved (IVM 4,9 oocytes versus ICSI 5,9 oocytes) . The mean number of MFII oocytes after collection of IVF/ICSI oocytes were 5,1 versus 2,1 for the IVM oocytes after 28 h maturation (P<0,01) however finally we found no significant change in rate of displacement of the spindle apparatus in the in vivo matured oocytes compared to the in vitro matured oocytes. (P > 0.05).



Conclusion

- The position (and morphology) of the spindle in MFII oocytes after IVM (94%) and after ICSI (91%) is normal
- MFII oocytes derived from in vitro maturation or in vivo maturation are not significantly different concerning cleavage rate



What about the endometrium

Endometrial lining and development after IVM.

7 days after oocyte collection a biopsy is taken.

Results:

A total of 10 biopsies were taken,

| | |
|----------------------|------------|
| Number in phase | 2 biopsies |
| Number out of phase | 7 biopsies |
| Number not evaluated | 1 biopsy |



Final remarks



IVM is approaching a pregnancy rate per egg retrieval of 30% in selected patient groups

Studies on the endometrial compartment is urgently needed

But:

| | |
|--|----------|
| Baby take home rate | 24/30 |
| Multiple pregnancy rate | 1/1 |
| OHSS risk | 0/10 |
| How are the offsprings doing ? | 1/1 |
| Cancellation rate | 30/25 |
| Cost for medication | 1/20000 |
| Lab cost | 160/1000 |
| Inteferece with daily life for the patient | 1/4 |
| Space for improvement : | yes |
| Cytoplasmic maturation | |



An emerging demand for cryopreservation of human oocytes either due to legislation or demands from the women due to clinical conditions is now a fact.

In this aspect IVM as a tool for providing immature or mature oocytes is obvious.



Roger G. Gosden

There is little debate about the desirability of human oocyte ("egg") banking but plenty of discussion about its prospects. Egg banking is needed by young cancer patients before they undergo potentially sterilizing treatment and is a desirable alternative to in vitro fertilization and embryo cryopreservation. However, egg banking is inefficient—oocytes are sensitive to chilling, often fail to survive freeze-thawing, and are susceptible to cytoskeletal damage and aneuploidy. Currently, even the most optimistic success rates offer patients only a slim chance of pregnancy if few oocytes are available. Ultra-rapid freezing with vitrification may offer advantages over conventional equilibrium cooling protocols and needs to be investigated further. Likewise, freezing immature oocytes followed by in vitro maturation offers practical and theoretical advantages, but this method is still inefficient. Nevertheless, all these technologies are improving, and egg banking will eventually become an option for patients seeking fertility preservation. [J Natl Cancer Inst Monogr 2005;34:66-3]



Oocyte vitrification—Women's emancipation set in stone

The techniques of vitrification of oocytes and the subsequent warming process being used today are now producing results far superior to the results that are obtained with slow-freezing techniques, and it would seem that this is the method of female fertility preservation that will be widely used in the near future. The reported success of the use of this method should stimulate a renewed debate on oocyte storage for fertility preservation without a medical indication. (Fertil Steril® 2008; ■ ■ ■ ©2008 by American Society for Reproductive Medicine.)

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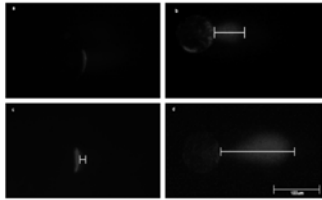
Table 3. Applications of oocyte and ovarian freezing

- Improve the efficiency of IVF
- Alternative to embryo freezing
- Oocyte preservation for patients with ovarian hyperstimulation syndrome
- Oocyte donation programme
- The treatment of congenital infertility disorders
- Prevent fertility loss through surgery
- Treatment of premature ovarian failure



FIGURE 1

DNA fragmentation analysis of mouse oocytes based on the length of the comet tail (magnifications, $\times 400$). (a) Fresh in vivo-matured oocyte. (b) Fresh in vitro-matured oocyte. (c) Vitrification of in vivo-matured oocyte. (d) Vitrification of in vitro-matured oocyte. The direction of electrophoresis was from left to right and the comet tail containing the DNA fragments was stained by ethidium bromide. Scale bar, 100 μm .



Wang. Vitrification of in vitro-matured oocytes. Fertil Steril 2007.



TABLE 3

Comparison of aneuploidy rates in in vivo- and in vitro-matured mouse oocytes after vitrification (3 replicates).

| Groups | Treatment | No. of oocytes analyzed | No. of oocytes with (%) | |
|------------------|---------------|-------------------------|-------------------------|------------|
| | | | Haploidy | Aneuploidy |
| In vivo-matured | Fresh | 105 | 99 (94.3) | 6 (5.7) |
| In vitro-matured | Fresh | 100 | 91 (91.0) | 9 (9.0) |
| In vivo-matured | Vitrification | 86 | 78 (90.7) | 8 (9.3) |
| In vitro-matured | Vitrification | 114 | 102 (89.5) | 12 (11.5) |

Note: There were no significant differences in the same column ($P < .05$).

Wang. Vitrification of in vitro-matured oocytes. Fertil Steril 2007.



TABLE 2

Clinical outcomes following vitrification of oocytes obtained from ovarian stimulation (OS) and in vitro maturation (IVM) cycles.

| | OS group | IVM group | P value |
|--|--------------------------|-----------------------|-----------------|
| Patients who underwent thawing and embryo transfer | 38 | 20 | |
| Mature (M2) oocytes retrieved | 299 | 8 | |
| Immature (O1) oocytes retrieved | 91 | 290 | |
| Oocyte maturation rate after IVM (\pm SD) | 70.3 \pm 20.0 | 87.3 \pm 19.3 | NS |
| Oocytes matured in vitro | 64 | 209 | |
| M2 oocytes vitrified and thawed (mean \pm SD) | 463 (2.2 \pm 5.7) | 215 (0.8 \pm 5.9) | NS |
| Oocytes survived (mean \pm SD) | 383 (81.4 \pm 22.8) | 148 (67.5 \pm 26.1) | $< .001^*$ |
| Oocytes fertilized (mean \pm SD) | 287 (75.6 \pm 22.5) | 98 (66.2 \pm 19.9) | $< .05^*$ |
| Embryos transferred (mean \pm SD) | 132 (35.5 \pm 1.5) | 64 (32.2 \pm 1.5) | NS |
| Cumulative embryo scores (mean \pm SD) | 38.4 \pm 23.3 | 20.0 \pm 13.8 | $< .05^*$ |
| Implantation per embryo (mean \pm SD) | 25/132 (19.1 \pm 25.8) | 4/64 (6.8 \pm 24.1) | $> 0.1^*$ |
| Pregnancy rate per cycle started (%) | 19 (50) | 4 (20.0) | NS ^b |
| Biochemical pregnancy | 2 | 0 | |
| Clinical pregnancy rate per cycle started (%) | 17 (44.7) | 4 (20.0) | NS ^b |
| Miscarriages | 2 | 0 | |
| Singleton pregnancies | 9 | 4 | |
| Twin pregnancies | 5 | 0 | |
| Triples pregnancies | 1 | 0 | |
| Live-birth rate per cycle started (%) | 15 (39.5) | 4 (20.0) | NS ^b |
| Newborns | 22 | 4 | |

Note: NS, not statistically significant.

*Mann-Whitney test.

^bFisher exact test.

Chen. Vitrification of in vitro-matured oocytes. Fertil Steril 2008.



TABLE 3

Obstetric and perinatal outcomes following vitrification of oocytes obtained from ovarian stimulation and in vitro maturation cycles.

| Group characteristics | OS | | IVM |
|-------------------------------------|-------------------------------|--|-------------------------------|
| | Singleton pregnancies (n = 9) | Multiple gestation pregnancies (n = 6) | Singleton pregnancies (n = 4) |
| Mean gestational age (weeks + days) | 39 + 1 | 36 + 4 | 39 + 3 |
| No. of delivery 34-37 weeks (%) | 0 | 4 (66.7) | 0 |
| No. of delivery <34 weeks (%) | 0 | 0 | 0 |
| | Singleton newborns (n = 9) | Multiple gestation newborns (n = 13) | Singleton newborns (n = 4) |
| Mean birth weight (g ± SD) | 3193.7 ± 376.8 | 2277.9 ± 395.7 | 4049.3 ± 413.7 |
| No. of LBW (1500 to 2500 g) (%) | 0 | 9 (69) | 0 |
| No. of VLBW (<1500 g) (%) | 0 | 0 | 0 |
| Males | 7 | 4 | 2 |
| Females | 2 | 9 | 2 |

Note: LBW: low birth weight; VLBW: very low birth weight.

Chair. Vitrification of in vitro matured oocytes. *Fertil Steril* 2008.



TABLE 1

Clinical outcomes of IVF-ET programs using vitrified/warmed human mature oocytes vitrified by slush nitrogen (SN₂) from stimulated cycles.

| | |
|--|--|
| No. of patients | 28 |
| No. of vitrified oocytes | 426 |
| No. of warmed oocytes | 354 |
| No. of survived oocytes (%) ^a | 302 (85.1 ± 2.9, ^b 79.2-90.9 ^c) |
| No. of microinjected oocytes | 218 |
| No. of fertilized oocytes (%) | 168 (77.4 ± 3.5, ^b 70.2-84.7 ^c) |
| No. of cleaved embryos (%) | 158 (94.3 ± 2.1, ^b 90.1-98.5 ^c) |
| No. of patients who underwent ET (%) | 30 (100) |
| No. of pregnancies (%ET) | 13/30 (43.3) |
| No. of pregnancies (%/patient) | 13/28 (46.4) |
| No. of multiple pregnancies | 3/13 (23.1) |
| No. of miscarriages (%) | 2/13 (15.4) |
| No. of deliveries/ongoing | 4 ^d /7 |
| No. of transferred embryos | 120 (4.0 ± 0.2, ^b 3.6-4.4 ^c) |
| Implantation rate (%) | 17 (14.2) |

^a No. of intact oocytes after warming (%vitrified oocytes).

^b Mean ± SEM.

^c 95% confidence interval.

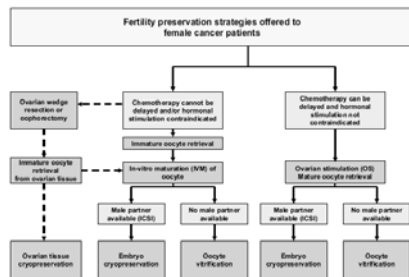
^d Three singletons (male/male/female), one twin (male/male).

Chair. Human oocyte vitrification using SN₂. *Fertil Steril* 2007.

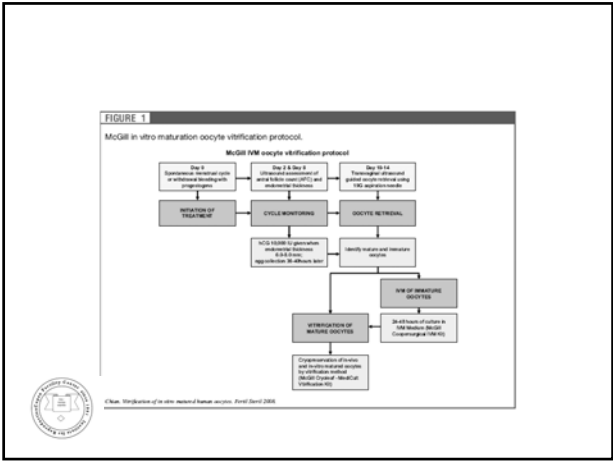


FIGURE 1

Preservation strategies offered to female cancer patients.



Chair. Vitrification of in vitro matured oocytes. *Fertil Steril* 2008.



Conclusion

IVM is still a method for special patients as the cancellation rate and implantation rate is less than in conventional IVF/CSI. However, - the quality of the IVM derived MFII oocytes are as good as other oocytes and this is the background for opening new therapeutic modalities as the fate of these gamete seems to produce healthy offsprings:

Oocyte banking
 Preserving fertility in women due to cancer treatment
 Preserving fertility in women due to late childbirth

All of this is a possibility due to IVM ova and vitrification