EGV
KLĪNIKA

CRYOPRESERVATION – CLINICAL ASPECTS

DR. SOLVITA FUNKA

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HISTORY

1978 – 1st baby after IVF.

England, R.G.Edwards, P.Streptoe


Evening News

Meet Louise, the world's first test-tube arrival

SUPERBABE

Wide-eyed Louise Brown pictured in hospital 18 hours after she was born. Today she's doing well. See Page Three

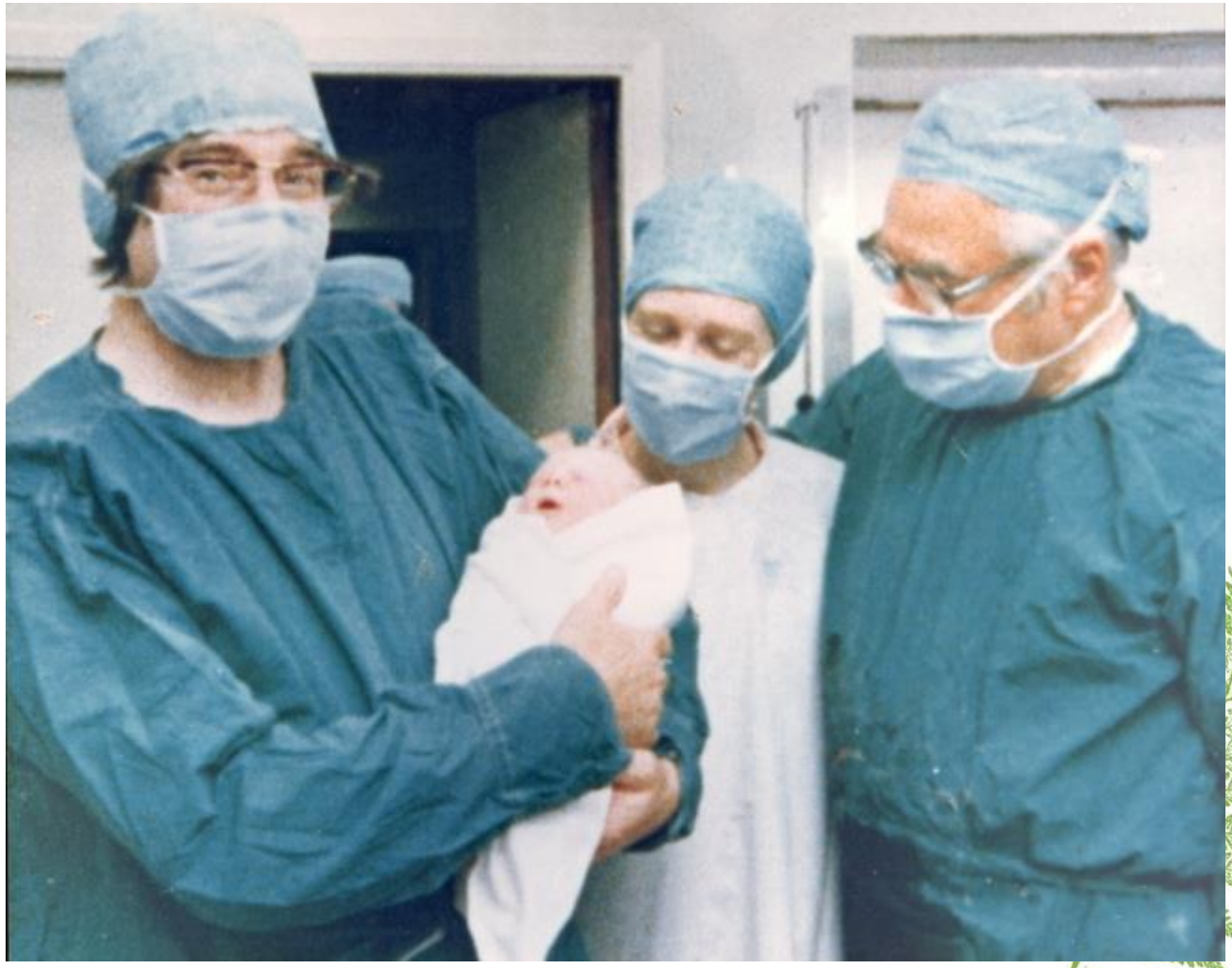
HISTORY

1978 – 1st baby after IVF.
England, R.G.Edwards, P.Streptoe

1983 – 1st baby after Crio ET.
Australia

1993 – 1st pregnancy after ICSI.
Netherlands

1997 – 1st baby after IVF in Latvia.
Daina Majore, Voldemārs Lejiņš



CONTENT IN BRIEF

- Cryopreservation method - slow freezing, vitrification
- Embryo vitrification
- Embryo cryopreservation – When?
- Blastocyst ET
- Cleavage stage ET
- Perinatal outcomes
- Semen
- Testicular material
- Oocyte
- Ovarian tisue

EMBRYO VITRIFICATION - BENEFITS

- High embryo survival rate
- Lower risk of losing the opportunity for embryo transfer
- Higher blastulation rates
- Eliminate the formation of intra- and extra-cellular ice crystals in cells
- Prevent osmotic shock from ultra-rapid cooling
- Method is not increasing any adverse short-term health outcomes for the infants

EMBRYO CRYOPRESERVATION (CP) WHEN

- Cancelled embryo transfer (OHSS risk, endometrial bleeding, elevated serum progesterone levels on the day of triggering, any other unplanned events).
- Freeze-all strategy in high responders, reduces OHSS.
- Superior embryo-endometrium synchronization
- Lower ectopic pregnancy rates

BLASTOCYST (BT) FROZEN EMBRYO TRANSFER #1

- BT results in a higher implantation rate
- BT has an extremely high survival rate
- Better clinical pregnancy rate, than cleavage-stage transfer, with a smaller number of transferred embryos
- Extended culture and transfer of blastocysts increase the synchronization of the endometrium
- Permits the selection of more advanced embryos considered best suited for transfer

BLASTOCYST (BT) FROZEN EMBRYO TRANSFER #2

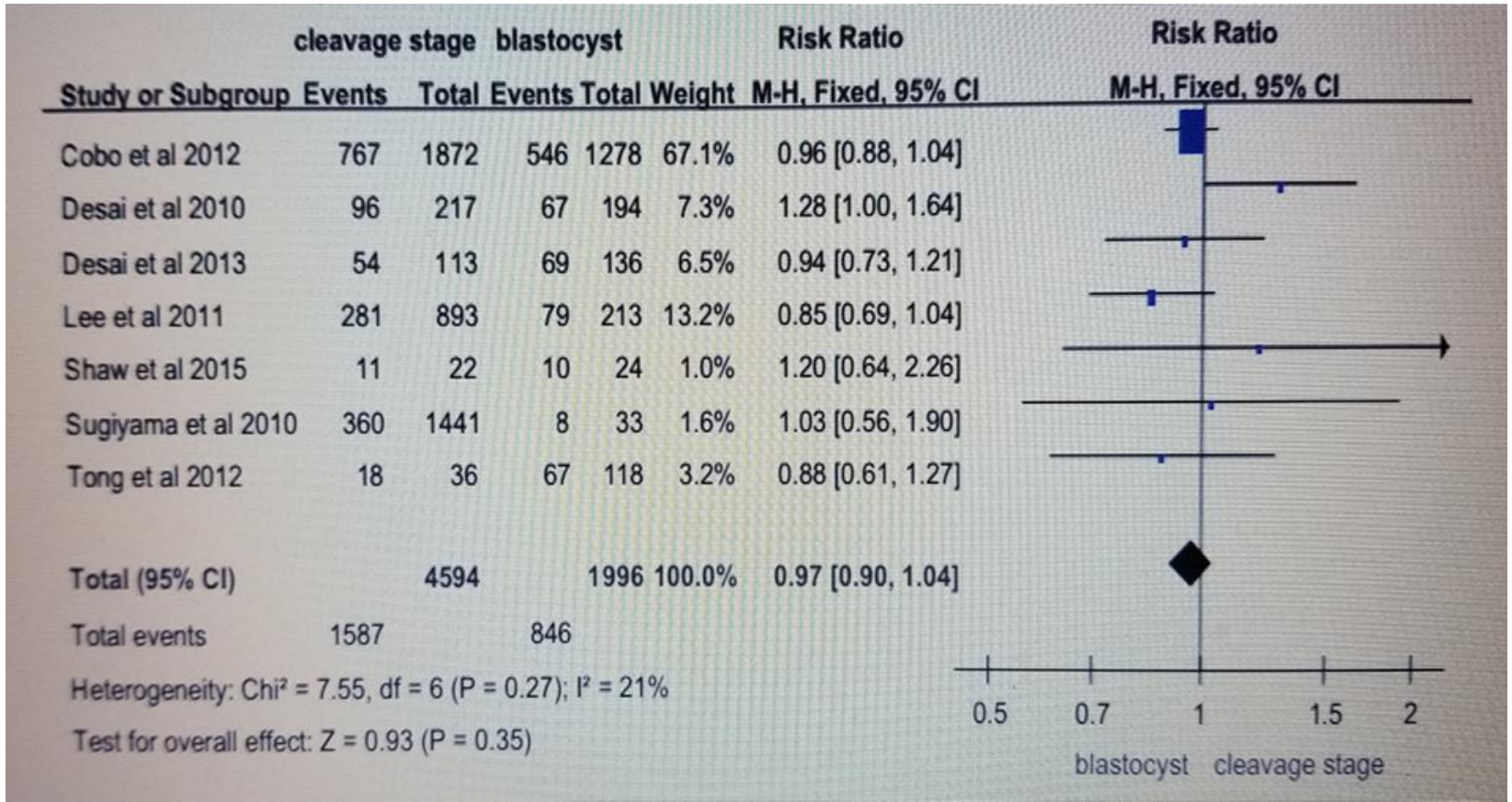
- BT decreases uterine contractility, thus reducing the chance of embryo expulsion
- BT prevents premature contact with an altered uterine environment after controlled ovarian stimulation, as supraphysiological concentrations of estrogen (E2) and progesterone (P) may influence endometrial receptivity

MINUSES – BLASTOCYST FET

- Cancelation rate in blastocyst stage is significantly higher than in the cleavage stage
- Lower freezing rates compared with the cleavage stage
- BT is also associated with higher monozygotic twin rates
- BT also increases the risk of premature delivery and babies with lower birth weights
- May contribute to the generation of epigenetic mutations in the offspring

CLEAVAGE-STAGE EMBRYO TRANSFER

- Cumulative clinical pregnancy rate for cleavage-stage embryos is higher than for blastocysts
- Smaller first trimester miscarriage rate in cleavage-stage embryo transfer group



Study or Subgroup	cleavage stage Events	Total	blastocyst Events	Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Cobo et al 2012	767	1872	546	1278	67.1%	0.96 [0.88, 1.04]
Desai et al 2010	96	217	67	194	7.3%	1.28 [1.00, 1.64]
Desai et al 2013	54	113	69	136	6.5%	0.94 [0.73, 1.21]
Lee et al 2011	281	893	79	213	13.2%	0.85 [0.69, 1.04]
Shaw et al 2015	11	22	10	24	1.0%	1.20 [0.64, 2.26]
Sugiyama et al 2010	360	1441	8	33	1.6%	1.03 [0.56, 1.90]
Tong et al 2012	18	36	67	118	3.2%	0.88 [0.61, 1.27]
Total (95% CI)		4594		1996	100.0%	0.97 [0.90, 1.04]
Total events	1587		846			

Heterogeneity: Chi² = 7.55, df = 6 (P = 0.27); I² = 21%

Test for overall effect: Z = 0.93 (P = 0.35)

PRO CONS D3/D5

Data is inconsistent

- Hreinsson et al. demonstrated that embryo transfer is equally effective at the cleavage stage and the blastocyst stage
- De Vos et al. debated the benefit of day 5 versus day 3 single embryo transfer
- Clinical pregnancy and the ongoing pregnancy is higher in the group in which blastocysts were vitrified on day 5

PERINATAL OUTCOMES

- Pregnancy and live birth rates after cryopreservation close to or even higher compared with fresh cycles.
- Mean birth weight and mean gestational age is higher in singletons born after FET (frozen ET)
- Singletons born after FET had a lower rate of Low birth wait (LBW) and pre-term birth (PTB)
- Post-term birth is higher after FET
- FET had a higher risk of Large for gestation age (LGA)

WENNERHOLM 2013, LARGE NORGIC COHORT STUDY

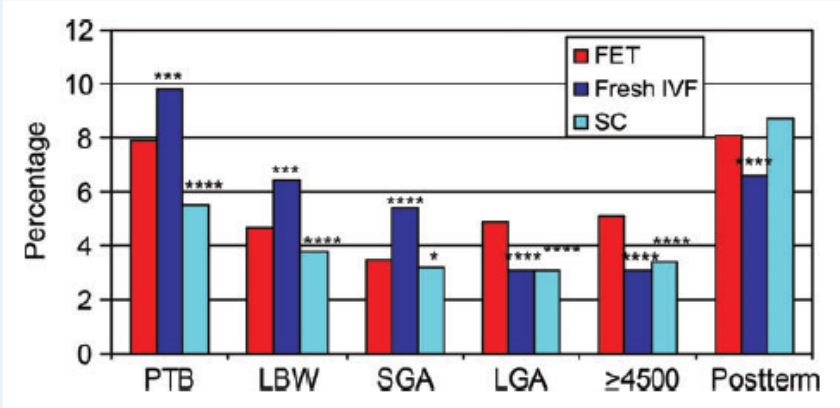


Figure 3 Distribution of gestational age and birthweight in children born after FET, fresh IVF (IVF, ICSI, IVF/ICSI) and spontaneous conception (SC). FET versus fresh IVF: PTB: $P = 0.0003$, LBW: $P = 0.0007$, SGA: $P < 0.0001$, LGA: $P < 0.0001$, ≥4500 g: $P < 0.0001$, post-term birth: $P < 0.0001$ (adjusted P values). FET versus SC: PTB: $P < 0.0001$, LBW: $P < 0.0001$, SGA: $P = 0.02$, LGA: $P < 0.0001$, ≥4500 g: $P < 0.0001$ (adjusted P values). PTB, preterm birth; LBW, low birthweight; SGA, small for gestational age; LGA, large for gestational age; ≥4500, birth weight ≥4500 g; postterm, ≥42 weeks. * $P < 0.05$, *** $P < 0.001$, **** $P < 0.0001$.

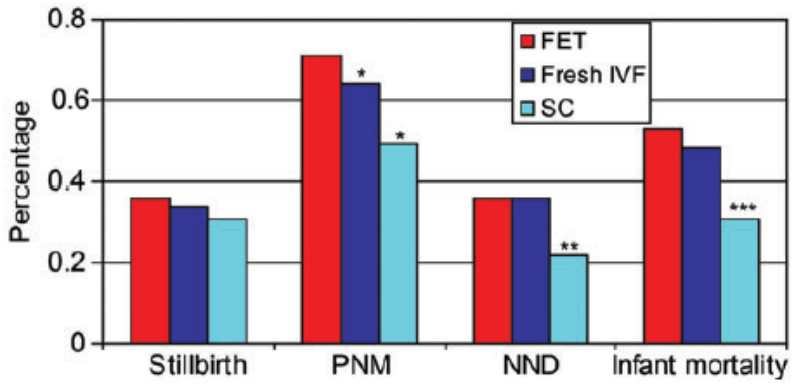
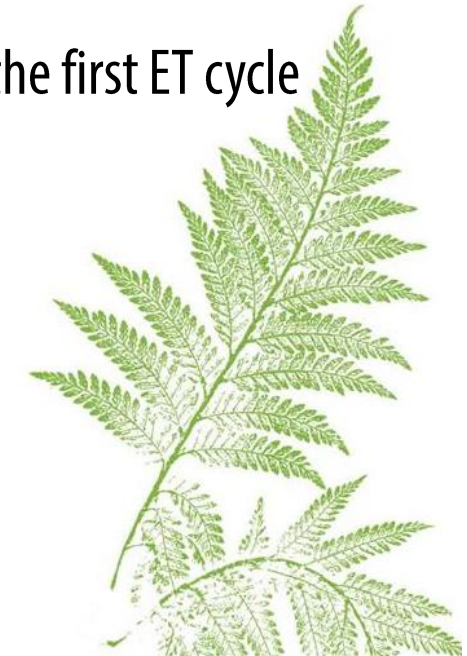


Figure 4 Distribution of stillbirth, perinatal mortality (PNM), neonatal death (NND) and infant mortality in children born after FET, fresh IVF (IVF, ICSI, IVF/ICSI) and SC. FET versus fresh IVF: PNM (≥28 weeks): $P = 0.02$ (adjusted P-value). FET versus SC: PNM (≥28 weeks): $P = 0.03$, NND: $P = 0.004$, infant mortality: $P = 0.0002$ (adjusted P-values). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

FOR WHOM?

- Tong et al. found that blastocyst culture and transfer should be offered primarily to younger patients (less than 35 years of age) with a better prognosis, in tandem with blastocyst vitrification
- Assisted hatching improves the chances of implantation rate during assisted reproduction
- Zhu et al. showed that BT with assisted hatching has a higher implantation rate compared to cleavage-stage embryos in vitrification cycles
- For high responders, the freeze-all strategy with thawed ET achieved a significantly higher OPR in the first ET cycle and a higher cumulative OPR than the fresh ET strategy
- Setting patient selection criteria for cryopreservatione of embryos



SEMEN – SPERMATOZOA

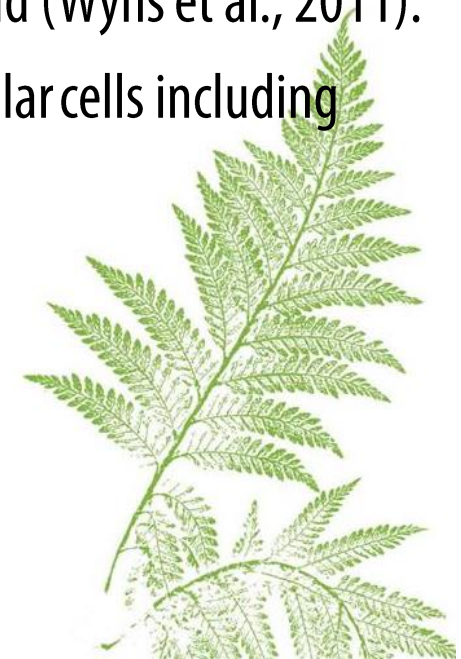
- First pregnancy with cryopreserved spermatozoa 1953 (IUI)
- Cryopreservation of spermatozoa is routinely used to preserve fertility in men (Sharma, 2011).
- Cryopreservation of sperm, first line fertility preservation treatment in adolescents (Daudin et al.,2015).

WHEN?

- Oncology
- Planing of treatment witch can lead to worstning of sperm quality
- Benign illness
- Autoimune – Polyarthritis, LED, Crona desise, Ulcerative colitis, Uveitis, Multiple sclerosis, Scleroderma, Psoriasis and other
- Before organ transplantation
- Acute paraplegia (loss of ejaculation 95%, sperm quality deterioration)
- Before a vasectomy or other surgery that may affect fertility
- Other causes of deterioration of sperm quality (varicocell, Klinefelter syndrome)
- “SOCIAL FREEZING”

FERTILITY PRESERVATION IN PREPUBERTAL BOYS AND ADOLESCENTS

- Semen can be cryopreserved for adolescent boys in more than 80% of cases (Kliesch et al., 1996; Bahadur and Raiph, 1999; Bahadur et al., 2006;Van Casteren et al., 2008a; Menon et al., 2009; Daudin et al., 2015).
- Semen samples can be obtained from boys from the age of 12 years onwards (Bahadur et al., 2006)
- Testicular sperm extraction (TESE) and storage
- Testicular tissue cryopreservation of prepubertal and peripubertal boys material of up to 16 years old (Wyns et al., 2011).
- Experimental techniques of cryopreservation of testicular tissue or suspensions of immature testicular cells including spermatogonial stem cells (SSCs)



PROBLEMS – OUTCOME #1

- No international guidelines for the duration of storage of spermatozoa, whether ejaculated or testicular
- If there are fewer than 0.1 × 106 sperm/ml present in the semen sample on freezing, then the success of semen cryopreservation is likely to be significantly reduced
- Successful IUI with samples stored for cancer patients range from 5 to 16% of patients provided that semen quality is high post-thaw. (Van Casteren et al., 2008)
- When IVF or ICSI are applicable using cryopreserved spermatozoa success rates are comparable to standard
- IVF and ICSI procedures in infertile couples. Depending on the centre, pregnancy rates of 23–57%.

(Agarwal et al.,2004; Schmidt et al., 2004; Hourvitz et al., 2008; Van Casteren et al., 2008b; Freour et al., 2012).

PROBLEMS – OUTCOME #2

- Educating key staff, capable of initiating discussions on fertility preservation is vital to the success of fertility preservation strategies (Nagel and Neal, 2008).
- In vitro maturation of round spermatids from adult testicular tissue has already led to the birth of healthy offspring (Tesarik et al, 1999)
- Fertilization competence of immature haploid cells retrieved from peri-pubertal tissue still remains to be proven
- Fertility restoration in animals have not yet been successfully translated to humans
- Ethical and legal questions?

OOCYTE CRYOPRESERVATION – ADVANTAGES

- Fertility preservation in women at risk of losing fertility due to oncological treatment, before the gonadotoxic treatment
- Premature ovarian failure, or chronic disease
- Alleviate religious and/or other ethical, legal, and moral concerns of embryo storage
- Helps overcome problems when husband is unable to produce a viable sperm sample or when spermatozoa cannot be found in the testis
- Makes "egg banks and/or egg donations" possible, eliminating donor-recipient synchronization problems
- Allowes women to postpone childbirth until a later time/age *(Social freezing)*
- Women with malignancy can achieve adequate ovarian response and similar oocyte parameters to those of women undergoing fertility preservation for non-cancer indications.

OOCYTE CRYOPRESERVATION – DIFFICULTIES

- The metaphase II (MII) oocyte has a very special structure

  Large size, very sensitive to low temperature, extremely fragile, high water content, low surface to volume ratio, presence of the spindle and other cell organelles, not optimal plasma membrane permeability to CPA and water, etc.) that leads to complex difficulties associated with its cryopreservation

- Hardening of the zona pellucida – which is a consequence of CP can adversely affect the normal fertilization process

OVARIAN TISUE CP #1

- Ovarian cortex cryopreservation and transplantation (OCT) is considered an experimental technique
- Lack of evidence regarding its efficiency and the risk of reintroduction of malignant cells
- More than 75 live births reported so far
- Few data concerning retransplantations
- Ovarian cortex CP considered as first option for FP when there is not enough time to complete ovarian stimulation for OV or in prepubertal patients
- No pregnancy occurred in the OCT group when the ovarian tissue was harvested beyond 36 years old (Diaz-Garcia 2017)

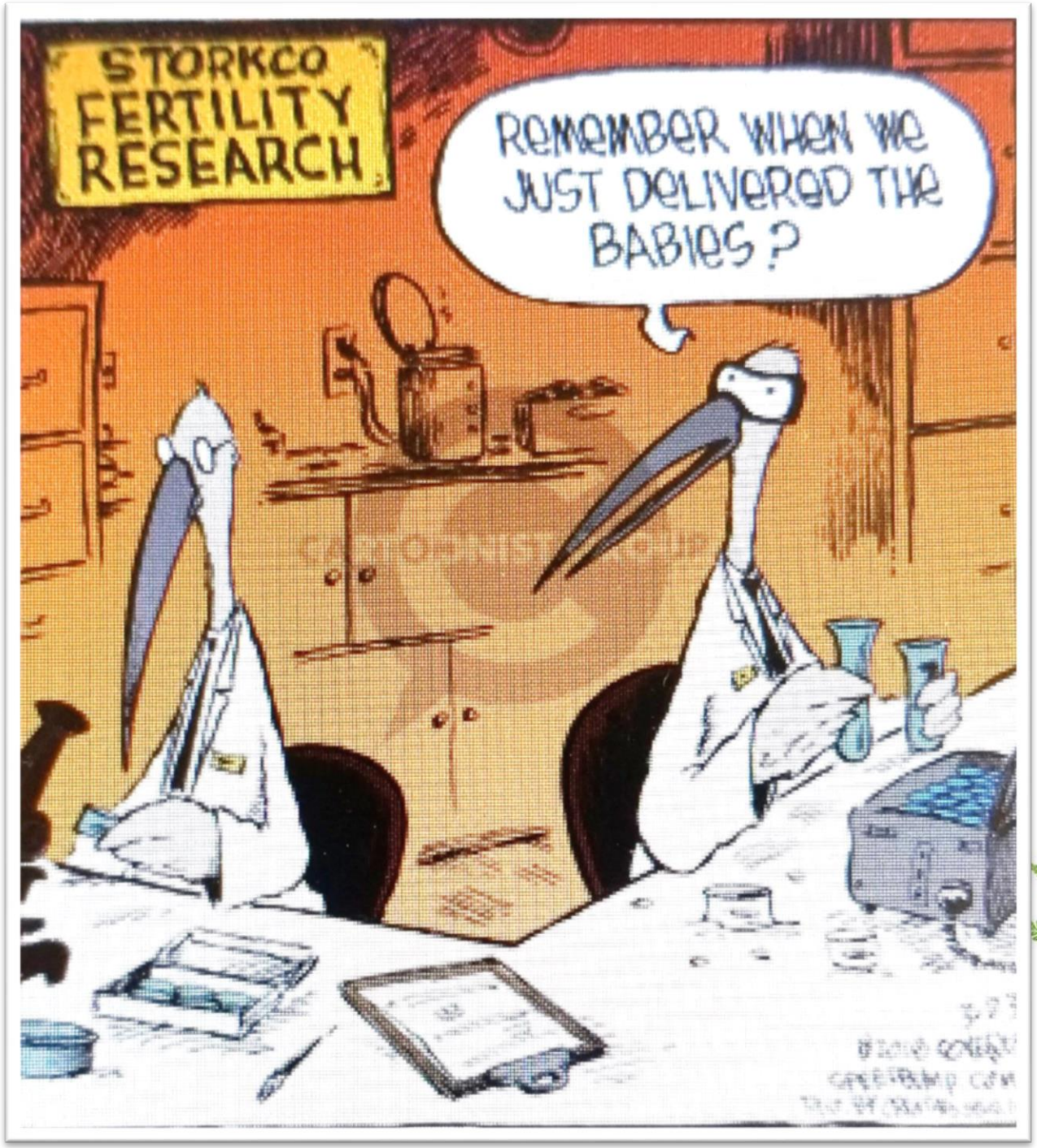
OVARIAN TISSUE CP #2

- Patients preserving for medical reasons (Ov medical LBR 28.6% vs. OV-social LBR, 50.0%; P<.001).
- LBRs after OCT in our series (18.2%)
- Cryopreservation and retransplantation of the ovarian tissue can cause dysfunctional folliculogenesis and asynchrony between the oocyte and the granulosa cells and alter oocyte morphology
- Restoration of ovarian function in 93.2% of patients after 3 months
- Spontaneous pregnancies in seven out of 15 patients

CONCLUSIONS

- High embryo survival rate after vitrification
- Blastocyst transfer results in a higher implantation rate
- Cryopreservation of sperm, first line fertility preservation treatment in adolescents
- Vitrification allows women to postpone childbirth until a later time/age (social freezing)
- Need to solve ethical and legal questions about tissue preserving.
- Tissue freezing research continues…

THANK YOU


STORKCO FERTILITY RESEARCH
REMEMBER WHEN WE JUST DELIVERED THE BABIES?