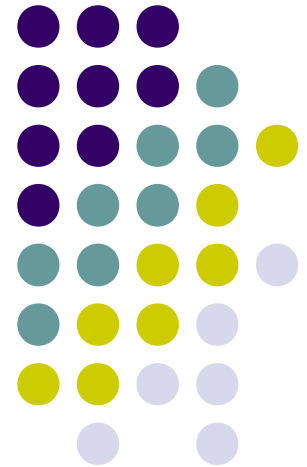
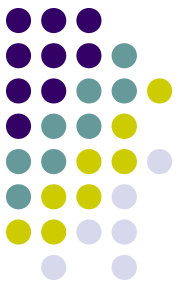


# Failed fertilization after clinical intracytoplasmic sperm injection

Reproductive BioMedicine Online  
2009 Vol. 19 No.2. 216–220

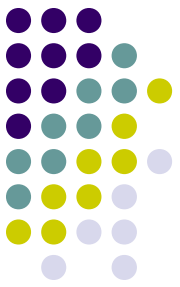
Present by R4 郭恬妮





# Introduction

- intracytoplasmic sperm injection (ICSI) → choice to overcome **male factor infertility** : *direct injection of a single spermatozoon into the cytoplasm of an oocyte*
- the major cause of infertility : sperm **motility** is impaired and **inability to penetrate the zona pellucida**
- ICSI is possible with spermatozoa obtained from ejaculation, microsurgical epididymal sperm aspiration, percutaneous epididymal sperm aspiration or testicular sperm extraction.



- **Total failed fertilization (TFF)** : failure of fertilization in all the mature oocytes and the term ‘failed fertilization’ refers to failure of fertilization in any mature oocyte.
- fertilization after ICSI is at about 70–80% ( *all ages with all the different sperm types*), TFF occurs in 5–10% of IVF cycles (*Mahutte and Arici, 2003*) and 1–3% of ICSI cycles (*Flaherty et al., 1998*).
- TFF after ICSI cycles : **low number of mature oocytes** or **oocyte activation failure**
- Considerable advances in artificial oocyte activation and recovery of spermatozoa from epididymis or testis that are suitable for ICSI help to avoid TFF

# Oocyte related factors

## *Oocyte morphology*

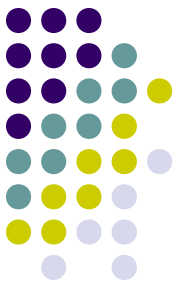


- **Poor oocyte morphology** is considered a major determinant of failed or impaired fertilization.
- Normal features of a healthy mature oocyte at metaphase II (MII) include:
  - *presence of a polar body,*
  - *a round even shape,*
  - *light colour cytoplasm with homogenous granularity,*
  - *a small perivitelline space without debris*
  - *a colourless zona pellucida*

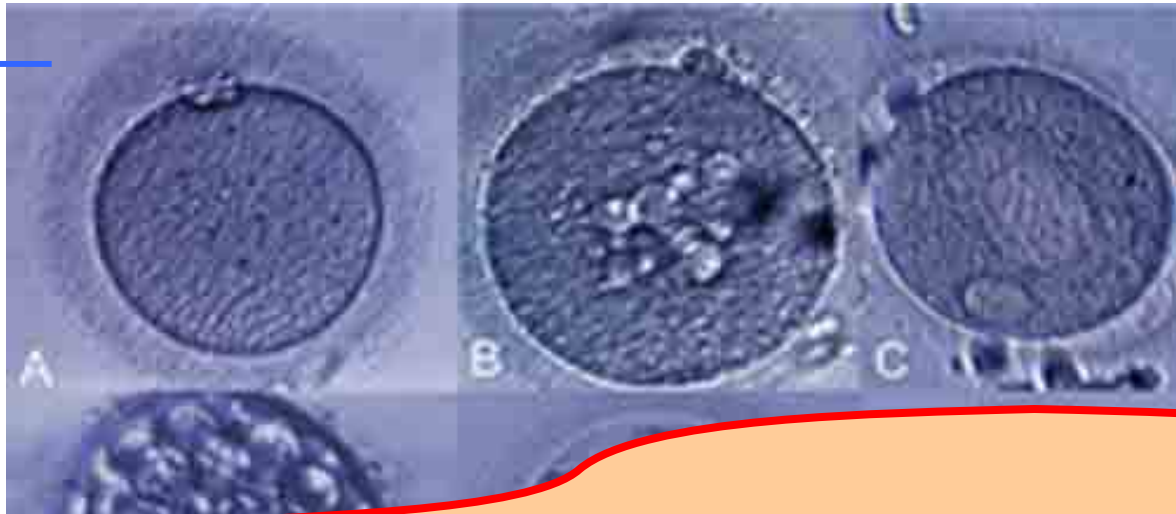


- In oocytes denuded for ICSI, the morphological structure and the nuclear maturity but **not cytoplasmic maturity** can be assessed in detail.
- The MII oocytes with apparently normal cytoplasmic organization may exhibit extra-cytoplasmic characteristics, such as:
  - increased perivitelline space
  - perivitelline debris
  - fragmentation of the first polar body (reduce developmental competence of the oocyte involved)
- It is common for extra-cytoplasmic and cytoplasmic dysmorphisms to occur together in the same oocyte (**Figure 1**).

Figure 1. Normal and abnormal morphology oocytes: (A) normal oocyte, (B, C) cytoplasmic vacuoles, (D, E) fragmentation, (F) perivitelline debris, and (G, H, I) abnormal zona pellucida and cytoplasm.



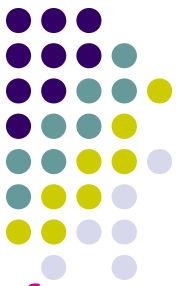
Normal



dysmorphic phenotypes → failed fertilization and aneuploidy → higher incidence of developmental failure

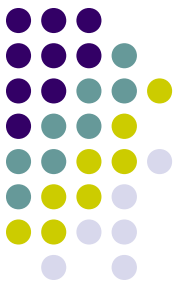
Meriano *et al.* (2001) : lower pregnancy and implantation rates when the transferred embryos with more than 50% dysmorphic oocytes.





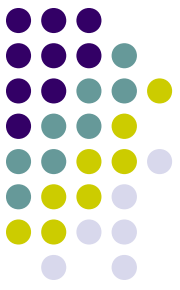
# Oocyte maturity

- One of the major causes of TFF after ICSI is **a low number of retrieved MII oocytes** (*Esfandiari et al., 2005a*).
- About **20%** of retrieved oocytes from ovarian stimulation cycles are **immature** → metaphase-I (MI) or germinal-vesicle (GV) stage in human IVF
- The injection of MI oocytes immediately after denudation → **high degeneration rate** ( increased fragility of the oolemma)
- The fertilization rate of retrieved MI oocytes is **lower** than retrieved sibling MI progressing to MII *in vitro* (25% compared with 62.2%, respectively).
- A **high rate of multinucleated oocytes** is also found in fertilized MI oocytes injected immediately after denudation (*Shu et al., 2007*).



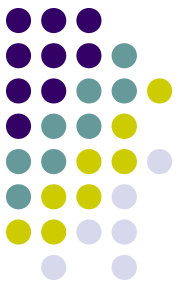
- In cases of **poor responders** and in patients with an **unsynchronized cohort** of follicles, the presence of **immature oocytes is frequent** after stimulation
- immature oocytes collected in stimulated cycles: under **stimulation with high doses of gonadotrophins** and are **exposed to human chorionic gonadotrophin before retrieval**.
- The nuclear maturation, cytoplasmic maturation and ensuing developmental capacity of these oocytes may **very well** (comparison with which collected from small antral follicles of unstimulated ovaries in the typical in-vitro maturation (IVM) procedure *(Sun et al., 2001).* )



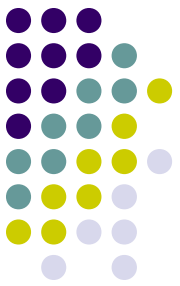


# *Oocyte activation*

- Oocyte activation is a complex series of events that results in the release of the cortical granules, activation of membrane-bound ATPase, resumption of meiosis, the formation of the male and female pronuclei with the extrusion of the **second polar body**. (activated started when the spermatozoon enters)
- In mammals:
  - MII (ovulated oocytes arrested ) → one spermatozoon contacts the oolemma and penetrates into the ooplasm → intracellular calcium oscillation occurs → increase in the concentration of Ca



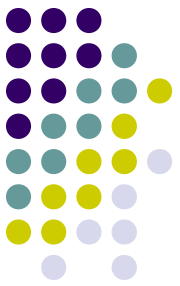
- The active component contained a protein moiety that possessed phospholipase C (PLC)-like activity capable of inducing production of IP<sub>3</sub>
- PLC activity was highly sensitive to calcium.
- A screen of expressed sequenced tags from testes identified a sperm-specific phospholipase C, PLC $\zeta$  → correlates with calcium activity in cytosolic sperm extracts (*Saunders, 2002*)



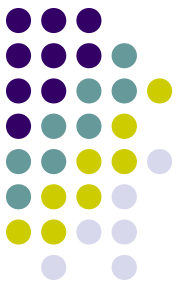
- Low fertilization rates after ICSI in patients with round-head spermatozoa, globozoospermia → **reduced ability of round-head spermatozoa** to activate the oocyte.
- Assisted oocyte activation combined with ICSI may overcome the infertility associated with globozoospermia.
- Besides, **cleavage rates** are also compromised and these **spermatozoa may lack normal centrosomes** (Battaglia *et al.*, 1997).



- Assisted oocyte activation aims to mimic the action of sperm penetration
- Some assisted activation treatments such as strontium chloride (*Cuthbertson et al., 1981*) and ionomycin (*Loi et al., 1998*), → increase in **intracellular free Ca concentrations** by the release of calcium from cytoplasmic stores
- A combination of calcium ionophore A23187 with puromycin stimulates the unfertilized oocytes 20–68 h after ICSI → activation rate of 91.2%, a cleavage rate of 64.7% and high-quality embryo rate of 44.1%.
- **Nearly all** activated embryos derived from 2PN/2PB had a **normal set of sex chromosomes and developed normally** (*Lu et al., 2006*).



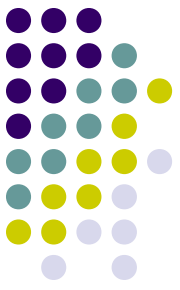
- An electrical field can generate **micropores in the cell membrane** of gametes and somatic cells to **induce sufficient calcium influx** through the pores to activate cytoplasm through calcium-dependent mechanisms (*Ozil, 1990*).
- The embryo formation rate was **80%** compared with 16% in the control group (*Manipalviratn et al., 2006*).
- fertilization rate was significantly higher in the electroactivated group (68%) as compared with that of the control (60%), **BUT!** a higher miscarriage rate was reported in the electroactivated group (five of 15 pregnancies) compared with the control (three of 33) (*Mansour et al., 2009*).



## *Poor ovarian response*

- The cut-off concentrations for the number of follicles or oocytes that define poor response vary widely from study to study.
- Various endocrine and ultrasonographic markers and dynamic tests to assess ovarian reserve have been evaluated.
  - basal FSH on cycle day 3
  - clomiphene citrate challenge test
  - inhibin B
  - Oestrogen
  - anti-Müllerian hormone
  - antral follicle counts
  - ovarian volume.

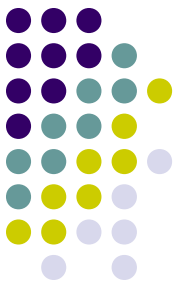
**none of these tests** has demonstrated a reliable predictive value and for many women poor ovarian response is not discovered until the first IVF cycle.



- One of the major contributing factors for TFF after ICSI is **three or less MII oocytes retrieved** (*Esfandiari et al., 2005a*).
- The rate of as the number of injected oocytes decreases fertilization failure increases (*Yanagida, 2004*).
- *Melie et al. (2003)*: a higher chance of having **no embryos** for transfer and **significantly lower pregnancy rates** when **less than five oocytes** are retrieved compared with cases with more than five oocytes

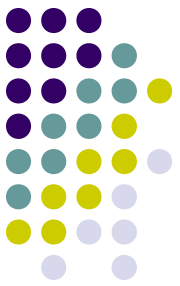
# Sperm-related factors

## *Sperm motility and progression*

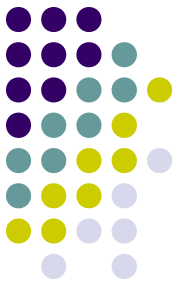


- Whether sperm movement is slow or rapid generally has no influence on ICSI results.
- However, injection of **immotile spermatozoa** usually results in **impaired fertilization** → possible that the spermatozoon may be **dead**.
- The most common practice to **select viable non-motile spermatozoa** for ICSI involves the hypo-osmotic swelling (HOS) test.
- In patients with 100% immotile spermatozoa, the HOS test is a useful method to **examine sperm viability**.
- It measures the functional integrity of the sperm membrane



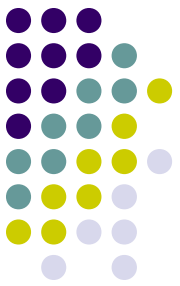


- Upon exposure of the spermatozoa to hypo-osmotic conditions, the intact semi-permeable barrier formed by the sperm membrane allows an influx of water and results in swelling of the cytoplasmic space and curling of the sperm tail fibres.
- **Only viable spermatozoa react to the HOS solution** since dead spermatozoa are unable to maintain the osmotic gradient.
- A **significantly greater fertilization and cleavage rate** after injection of spermatozoa selected using the HOS test is achieved in contrast to injection of randomly selected spermatozoa.



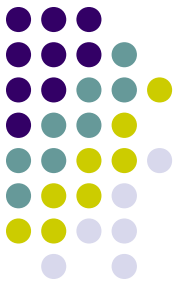
# *Sperm origin*

- Azoospermia: most severe form of male factor infertility, classified as ‘obstructive’ or ‘non-obstructive’.
- Obstructive:
  - the result of obstruction in either the upper or lower male reproductive tract
  - Sperm production may be normal but the obstruction prevents the spermatozoa from being ejaculated.
- Non-obstructive:
  - the result of testicular failure where sperm production is either severely impaired or nonexistent

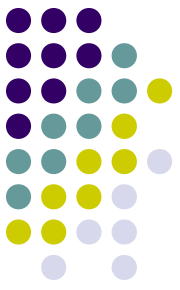


- The effect of cryopreservation of spermatozoa on ICSI outcome has been thoroughly studied.
- Current studies suggest that the use of fresh or frozen–thawed spermatozoa does not appear to affect ICSI outcomes *(Lewis and Klonoff-Cohen, 2005)*.
- Testicular tissue and epididymal spermatozoa can be **cryopreserved successfully** without markedly reducing subsequent fertilization and implantation rates and repeated testicular biopsy can be avoided without the risk of any decrease in the outcome *(Matyas et al., 2005)*.

# Sperm maturity



- Round spermatid nucleus injection (ROSNI) or round spermatid injection (ROSI) are methods in which precursors of mature spermatozoa obtained from ejaculated specimens or testicular sperm extraction are injected directly into oocytes.
- ROSNI has been proposed as a treatment for men in whom other more mature sperm forms (elongating spermatids or spermatozoa) cannot be identified for ICSI (*Saremi et al., 2002*).
- It is not widely performed and not as successful as ICSI and it is still an experimental procedure.

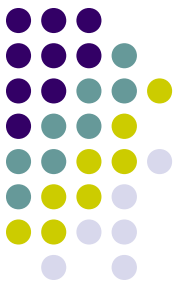


- Mouse round spermatids have **increased levels of DNA fragmentation** → interfere with fertilization.
- **Increased DNA damage** may occur because of **deficient sperm nuclear protamine to histone replacement** and **decreased nuclear condensation** in these immature spermatozoa
- Another major concern is genetic risk → severe to result in **meiotic arrest** during spermatogenesis may also have adverse effects on other normal cellular processes or other systemic manifestations.
- Patients who may be candidates for ROSNI should receive **careful and thorough pretreatment counselling** to ensure they are clearly informed of the limitations and potential risks of the procedure

# *Sperm structural defects*

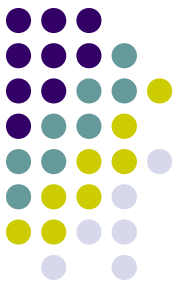


- Normal sperm ultrastructure correlates with positive IVF results
- Single structural defects → is of **genetic origin** and is generally transmitted as an autosomal recessive trait.
- Numerous defective genes are potentially involved in human isolated **teratozoospermia** (*FrancaVilla et al., 2007*).
- An in-depth evaluation of sperm morphology by transmission electron microscopy (**TEM**) can improve the diagnosis of male infertility and can give substantial information about the fertilizing competence of spermatozoa (*Kupker et al., 1998; Yu and Xu, 2004*).



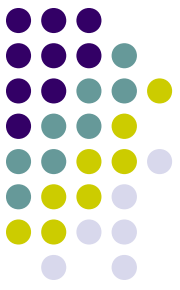
- **Acrosome agenesis** is most often associated with a spherical shape of the head and is usually defined as ‘round head defect’ or globozoospermia
- Globozoospermia results from perturbed expression of nuclear proteins or from an altered golgi–nuclear recognition during spermiogenesis.
- the fertilization rate after ICSI with round-head spermatozoa ranges from 0% to 37% (*Battaglia et al., 1997; Rybouchkin et al., 1997*).
- The most likely cause for failed fertilization after ICSI using round-head spermatozoa is **inability** of the spermatozoon **to activate** the oocyte

# Premature chromosomal condensation

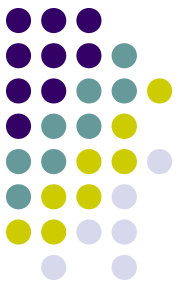


- When a cell with chromosomes in MII fuses with an interphase cell, the **nuclear membrane** of the cell in interphase **dissolves** and its **chromatin condenses** → **premature chromosomal condensation (PCC)** (*Johnson and Rao, 1970*).
- Chromatin analysis of human oocytes has revealed that sperm PCC is one of the prevalent causes of fertilization failure in both IVF and ICSI (*Schmiady et al., 1986*).
- The **failure of fertilization after ICSI** may result from either the **lack or deficiency of activating factors** in the spermatozoon or from the **lack of ooplasmic factors triggering sperm chromatin decondensation** (*Van Blerkom et al., 1994; Yanagida et al., 1999*).





- During normal spermiogenesis, **85% of histones** are replaced with **protamines** (*Balhorn, 1982*), → sperm chromatin condensation.
- A spermatozoon with a condensed nucleus is in the G1 stage when entering a MII oocyte and is protected from PCC because an active **maturation-promoting factor (MPF)** is **not capable of reacting with protamine-associated DNA**.
- Once sperm nuclear decondensation factors from the ooplasm enter the spermatozoon, the sperm head swells and sperm-associated oocyte-activating factor is released → This results in MPF inactivation (*Dozortsev et al., 1997*)

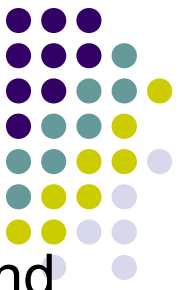


- During this time, protamines are slowly replaced by histones and cell cycle synchronization takes place.
- Sperm **PCC** has been associated with the type of **ovarian stimulation protocol** ( such as clomiphene citrate and human menopausal gonadotrophin stimulation ) may tend to recruit immature oocytes with immature cytoplasm (*Ma and Yuen, 2001*).
- **Immature cytoplasm** : a high incidence of PCC after insemination because of the **inability of the immature oocyte to undergo oocyte activation** (*Calafell et al., 1991*).

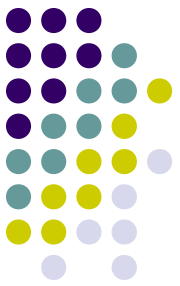
# Sperm DNA damage



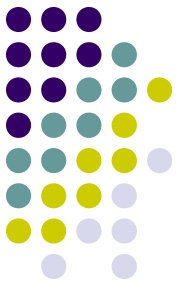
- **DNA damage in the male germ line** → associated with poor fertilization rates following IVF, defective preimplantation embryonic development and high rates of miscarriage and morbidity in the offspring, including childhood cancer
- **Activation of embryonic genome expression** occurs at the **4–8-cell stage in human embryo** → the paternal genome may not be effective until that stage.
- Therefore, a **lack of correlation** between elevated DNA strand breaks in spermatozoa and fertilization rates may occur before the 4–8-cell stage (*Twigg et al., 1998; Tesarik et al., 2004*).



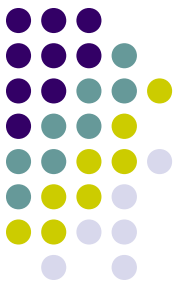
- **Nuclear DNA damage** in **mature spermatozoa** ( single-strand nicks and double-strand breaks ) → because of errors in **chromatin rearrangement** during spermiogenesis, **abortive apoptosis and oxidative stress** (*Lopes et al., 1998; Sikora et al., 2006*).
- Two tests have been most commonly reported as indicators of **sperm nuclear integrity**:
  - *terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling (TUNEL)*
  - *sperm chromatin structure assay (SCSA)*.
- The **TUNEL** technique labels single- or double-stranded DNA breaks, but does **not quantify DNA** strand breaks in a given cell.



- **SCSA**, a **quantitative** and **flow cytometric test**, measures the susceptibility of sperm nuclear DNA to acid-induced DNA denaturation *in situ*, followed by staining with acridine orange (*Evenson et al., 2002*).
- **SCSA accurately estimates the percentage of sperm chromatin damage** expressed as **DNA fragmentation index (DFI)** with a cut-off point of 30% to differentiate between fertile and infertile samples (*Potts et al., 1999*).
- A statistically significant difference was seen between the outcomes of ICSI versus IVF when **DFI was more than 30%** (*Bungum et al., 2007*).



- At present, there are two major strategies that may be considered for the treatment of men exhibiting high levels of DNA damage in their spermatozoa:
  - (i) selective isolation of relatively undamaged spermatozoa
  - (ii) antioxidant treatment (*Aitken et al., 2009*).



# *Procedural effects of the ICSI technique*

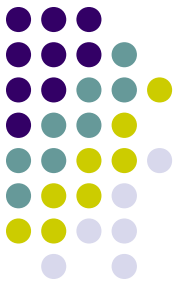
- The risk of oocyte damage by the **ICSI procedure** is low → may be related to
  - the **skill** of the person performing the injection procedure
  - the **quality and quantity of the gametes** used during the procedure (*Palermo et al., 1995*).
- One common technical failure is **not depositing the spermatozoon within the oocyte cytoplasm**.
- In this situation, the oocyte membrane may not have been broken during attempts to aspirate the ooplasm into the ICSI needle.

- Thus, the spermatozoon is deposited next to the membrane so that when the oolemma returns to its original position, the spermatozoon is **pushed out into the perivitelline space (Figure 2A)**, or is trapped inside a sac formed by the membrane (**Figure 2B**) (Esfandiari *et al.*, 2005a).

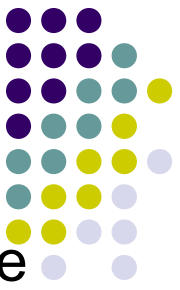


- (A) a spermatozoon trapped in perivitelline space (arrow),
- (B) a spermatozoon trapped in a membrane fold (arrow)
- (C) an atretic oocyte.





- The **degeneration of oocytes** after ICSI is often a result of a fault in the ICSI technique, e.g. an injection pipette that is too large or not sharp enough.
- Aspiration of the ooplasm is always used to make sure that the oocyte membrane is broken during injection.
- However, if the ooplasm is aspirated too much, degeneration of the oocyte frequently results (**Figure 2C**).

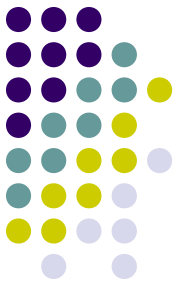


- Proper orientation of the polar body and needle position are also important, since improper positioning can damage or disrupt the metaphase plate during needle entry.
- disturbances in the nuclear spindle may dispose oocytes to aneuploidy or maturation arrest.
- During ICSI, the location of the first polar body is commonly used as an indication of the spindle position, with the assumption that they are located in close proximity.
- To avoid damage to the spindle, oocytes are injected at the 3 o'clock position with the first polar body at the 6 or 12 o'clock position.

# ICSI after previous ICSI cycle failure



- **Repeated ICSI treatment** can be useful or necessary because there is **a high possibility of achieving normal fertilization** if a reasonable number of oocytes with normal morphology are available and motile spermatozoa can be found.
- If there are no motile spermatozoa present in the first ejaculate, a second sample should be required followed by percutaneous epididymal sperm aspiration or testicular sperm extraction to obtain motile spermatozoa.

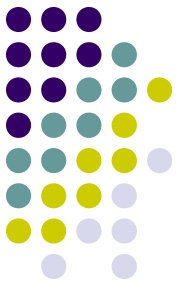


- **One-third** of the patients with **TFF** achieved pregnancy with their own oocytes in a subsequent ICSI cycle (*Esfandiari et al., 2005a*).
- Since follow-up ICSI treatment has been shown to result in fertilization in **85%** of cases, **repeated ICSI attempts are suggested in TFF** (*Flaherty et al., 1998; Rouzi and Amarin, 2002*).

# Options for patients after repeated ICSI cycle failure



- The adverse result of a failed ICSI cycle does not imply a hopeless prognosis for future ICSI treatment.



Thanks for your attention!