

Semen analysis in 21st century medicine: the need for sperm function testing

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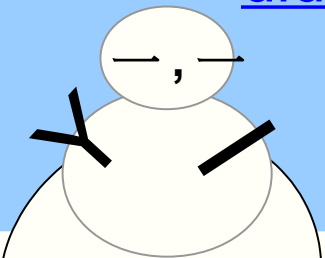
Presented by: Kitty Huang

Mar. 2nd, 2010



Introduction

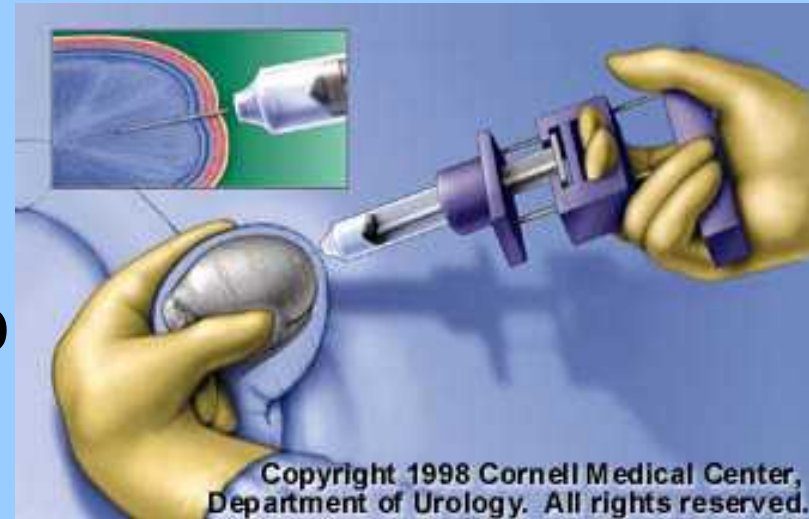
- With the advent of **intracytoplasmic sperm injection (ICSI)**, the evaluation and treatment of the infertile male radically changed.
 - Just a single spermatozoon was required
 - motility was not necessary
 - the normal biological processes of sperm capacitation, the acrosome reaction (AR), cumulus penetration, zona and ova binding, and penetration did not necessarily occur before fertilization.



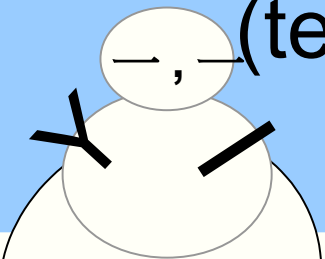
Introduction

- Sources of spermatozoa from men with non-obstructive azoospermia for ICSI-IVF:

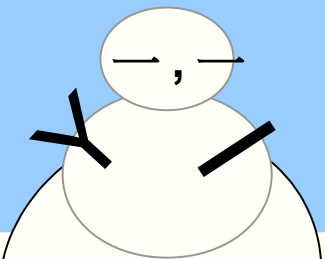
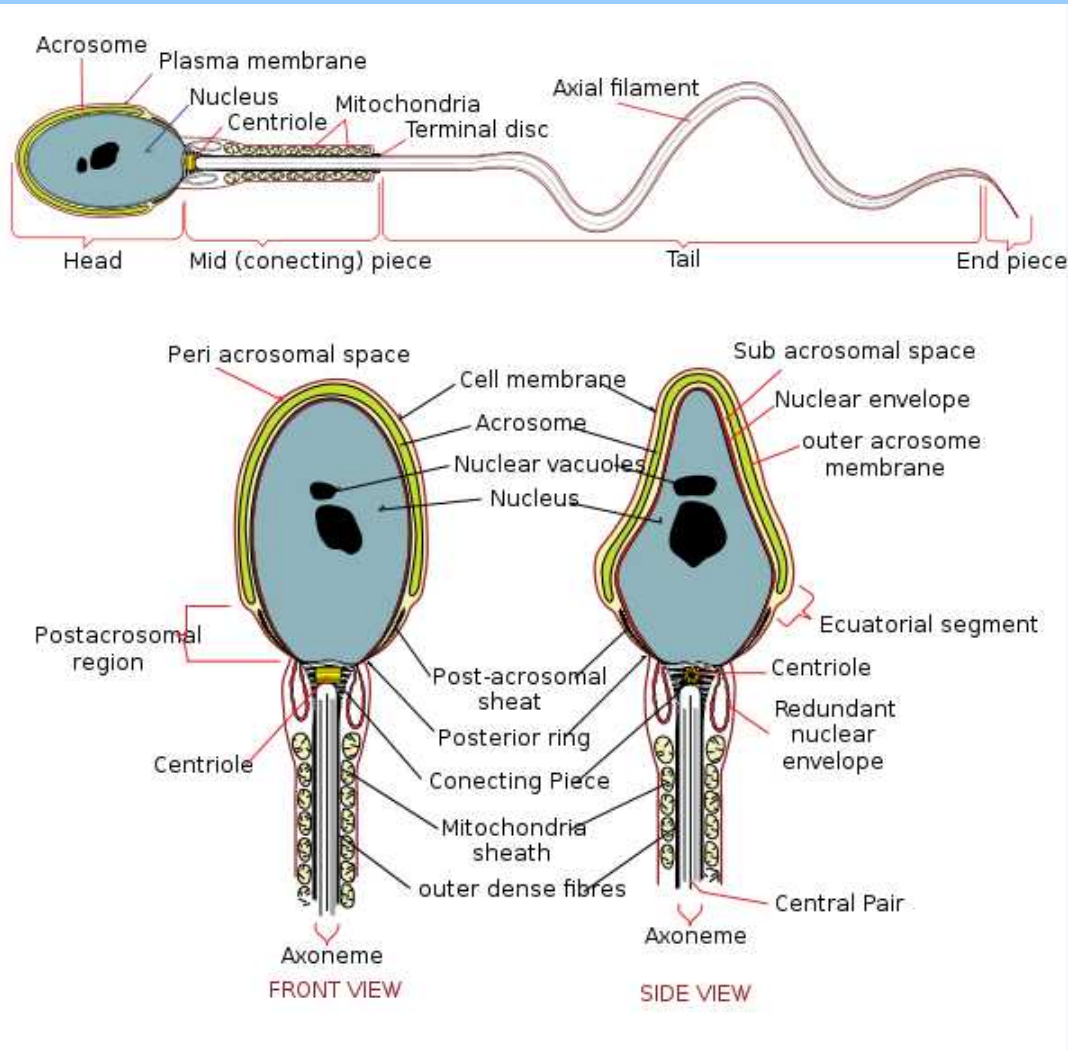
- ejaculated spermatozoa
- microsurgical epididymal spermatozoa aspiratio
- directly from the testis



(testicular sperm extraction)

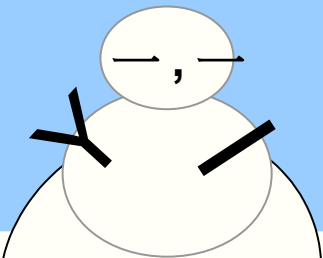


Introduction



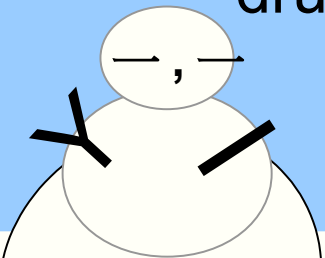
Introduction

- World Health Organization (WHO) Laboratory Manual for the Examination and Processing of Human Semen



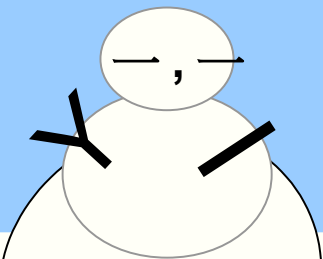
Introduction

- Routine semen analysis provides useful information concerning:
 - sperm production by the testis
 - sperm motility and viability
 - the patency of the male genital tract
 - the secretions of the accessory organs
 - ejaculation and emission
 - assessment of effects of environmental toxicants or drug studies

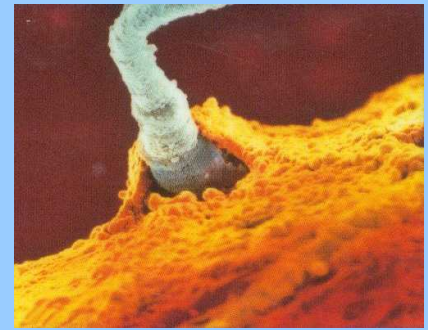


Introduction

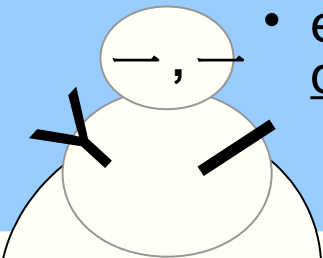
- Semen analysis
 - not a test of fertility
 - it provides no insights into the functional potential of the spermatozoon or to undergo the subsequent maturation processes
 - cannot distinguish the fertile from the infertile population, unless the man is azoospermic



Introduction

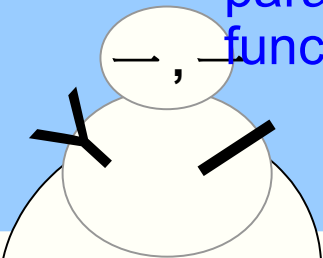


- Sperm function:
 - the ability of one spermatozoon to deliver the correct complement of chromosomes to an ovum
 - be produced in sufficient #, and exhibit normal motility and shape
 - capable of penetrating and passing through the cervical mucus, and through the uterus to the ampullae of the oviducts
 - undergoing capacitation, AR, binding and penetration of the zona pellucida, and ultimately the ovum
 - nuclear decondensation to deliver the appropriate haploid chromosome complement
 - events required for fertilization and early embryonic development



Introduction

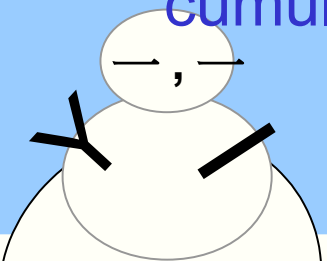
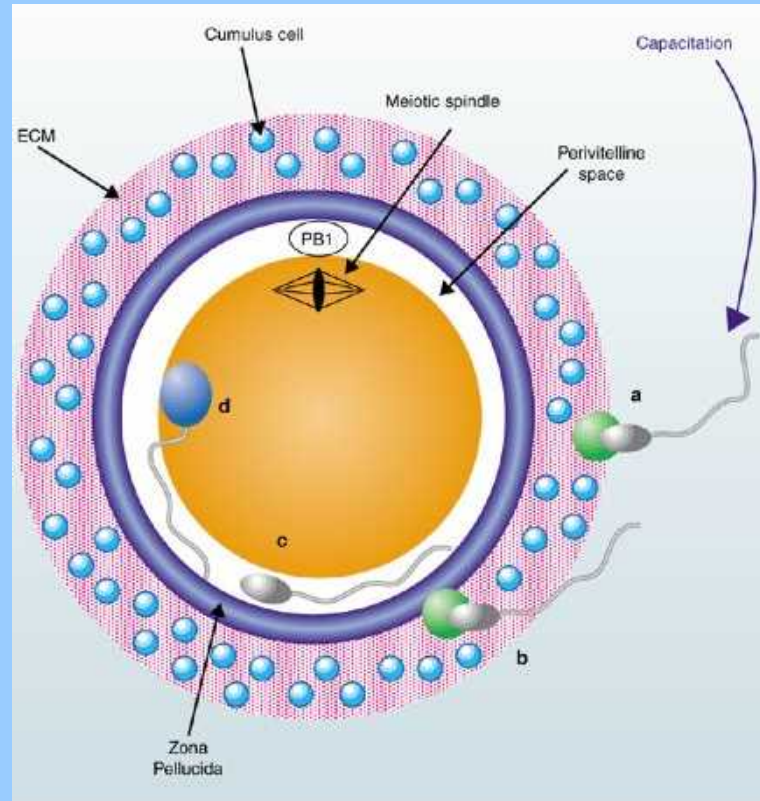
- Tests have been developed to identify abnormalities in these processes.
 - These tests can have applications in evaluations of potential toxins, as well as in clinical trials for drug testing.
- The clinical use of these tests ↓ significantly with the onset of ICSI, despite the fact that evaluation prior to treatment could:
 - prevent over treatment with the most advanced and costly technology
 - prevent unexpected IVF failure for men with normal semen parameters (as measured by the S/A), but with unrecognized functional sperm deficiencies.



Tests of sperm capacitation

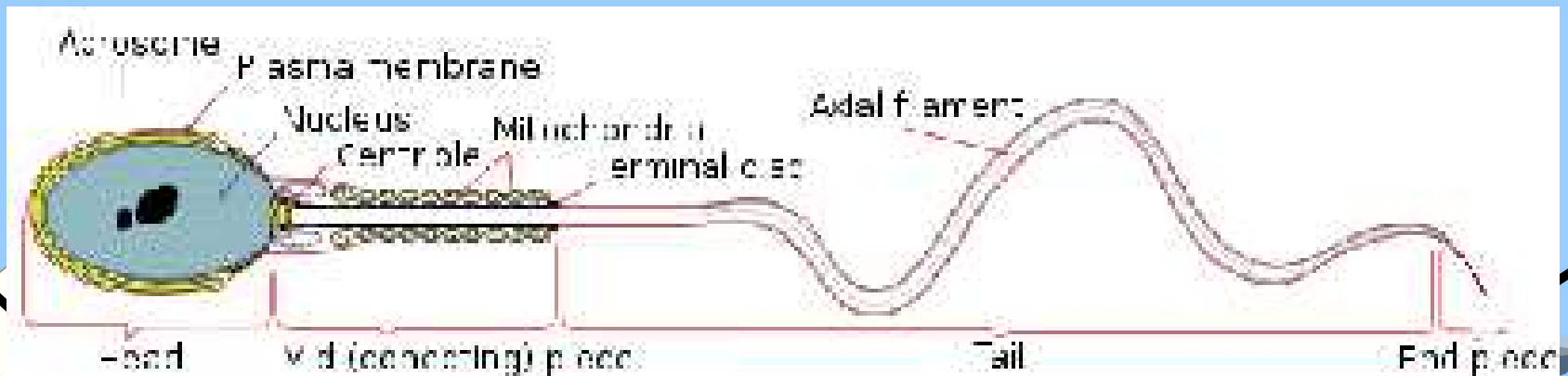
- **Capacitation**: a collection of changes in sperm function that occur generally in the female genital tract

– change in the membrane permeability to Ca ions, which induce a hyper motility of the sperm, a process that is thought to aid in penetration of the cumulus and zona pellucida

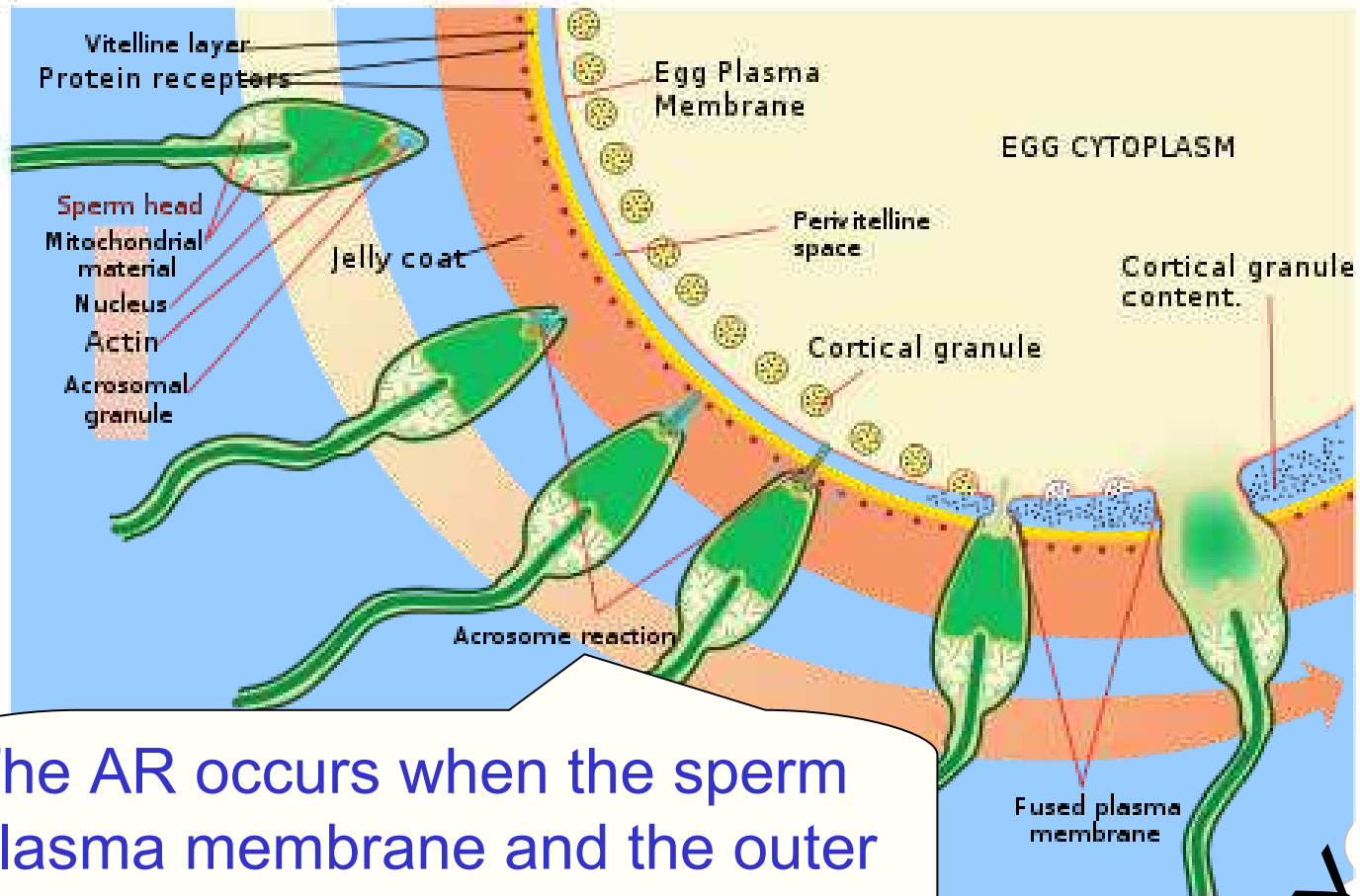


Tests of sperm capacitation

- AR test
 - measures the ability of the acrosome, essentially a modified Golgi apparatus limited by the inner and outer acrosomal membranes and located beneath the sperm plasma membrane, to release hydrolases (with **acrosin** being the predominant enzyme).



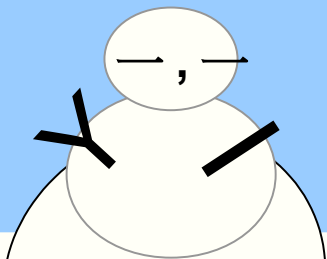
Tests of sperm capacitation – AR Test



The AR occurs when the sperm plasma membrane and the outer acrosomal membrane fuse.

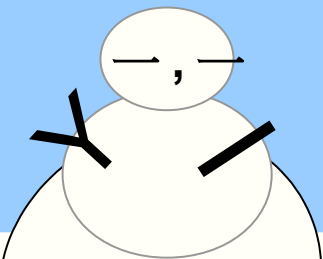
Tests of sperm capacitation – AR Test

- Biochemical agents, such as **calcium ionophore A23187** and **progesterone**, can induce the AR in vitro
 - allows an assessment of the maximal potential induction of AR



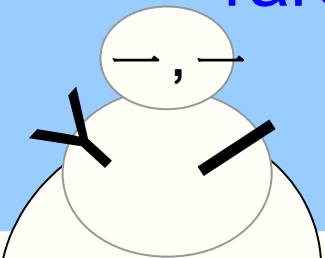
Tests of sperm capacitation – AR Test

- Studies have not compellingly shown that the AR assay results predict the likelihood of successful IVF, although some papers have reported a positive correlation.
- With the development of IVF–ICSI, however, this AR assay is rarely used.



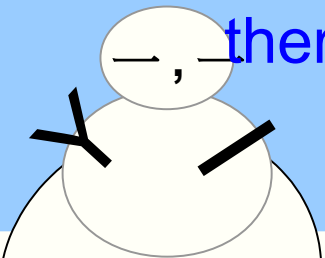
Tests of sperm capacitation – AR Test

- The AR assay can definitively identify globozoospermia
 - but this defect is already easily identified when the strict (now routine) morphology assessment is performed.
 - Electron microscopy remains the gold standard for globozoospermia (but this is rarely performed in the andrology laboratory).



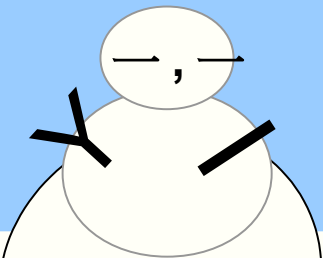
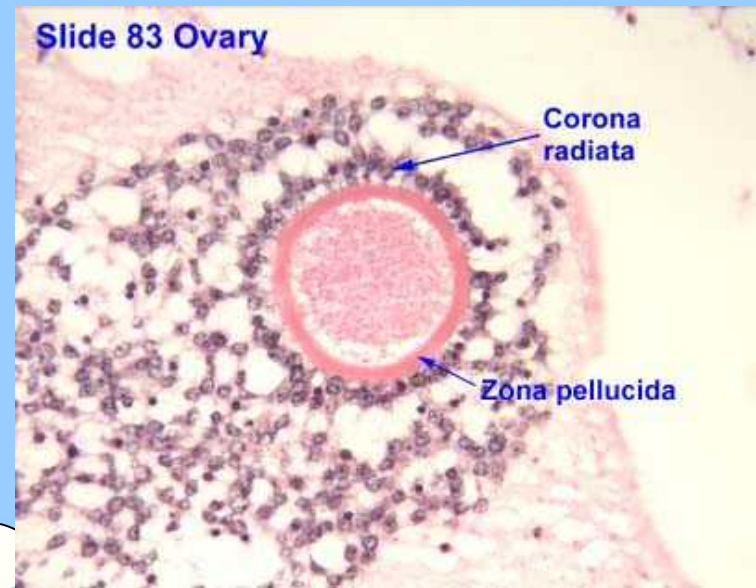
Tests of sperm capacitation – AR Test

- Whether quantitative measurement of **acrosin** is useful in the evaluation of the infertile male remains controversial.
 - Despite several reports stressing the usefulness of acrosin measurements, the test is not widely performed, and correlation with fertilization outcomes are difficult.
 - Rigorous, highly controlled, large-scale studies with appropriate positive and negative controls are therefore needed in this regard.



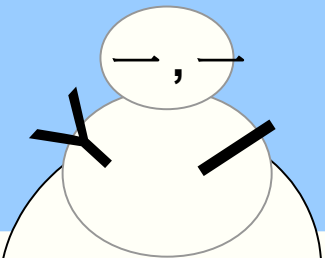
Tests of hemizona and zona-pellucida binding

- Sperm binding and penetration of the zona pellucida are critically important to fertility.
 - in couples with fertilization failure in IVF, spermatozoa sometimes failed to bind to the zona pellucida



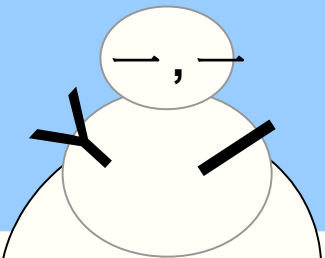
Tests of hemizona and zona-pellucida binding

- Burkman et al. developed a **hemizona assay**, designed to predict fertilization (or at least sperm–zonae interaction).
 - difficult for the average andrology or IVF laboratory to perform
 - no longer widely offered



Sperm penetration assay or sperm capacitation index or zona-free hamster oocyte penetration assay

- In 1976, Yanagamachi et al. made the remarkable observation that after removal of their **zona pellucida**, the hamster eggs are promiscuous—they allow spermatozoa from several species (including humans) to penetrate.



Sperm penetration assay or sperm capacitation index or zona-free hamster oocyte penetration assay

- Sperm penetration assay (SPA) using denuded hamster ova

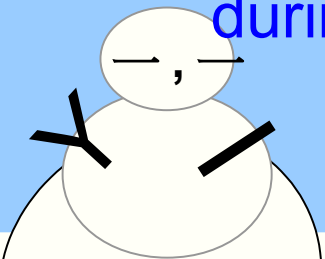
- to test sperm capacitation

The test provide some predictive information regarding the potential for ART success

- sperm should penetrate the egg

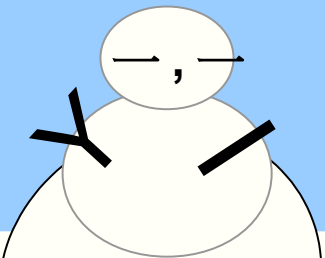
- sperm head

- decondensation in a manner similar to that occurring during the initial events of fertilization



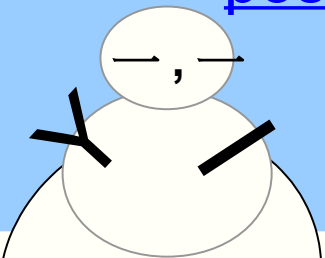
Sperm penetration assay or sperm capacitation index or zona-free hamster oocyte penetration assay

- Several modifications to this test were developed, but its predictive value was controversial.
 - Some laboratories reported a high correlation with IVF outcome and a low false-positive rate
 - others reported an unacceptable level of false negatives
 - the IVF procedure used would be modified.
 - higher # of sperm would be added to the ova
 - washing and sperm preparation techniques would be altered in an attempt to improve IVF outcome



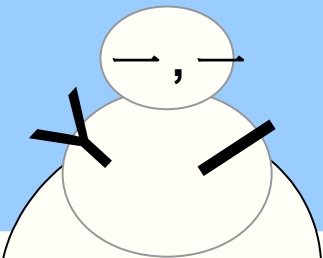
Sperm penetration assay or sperm capacitation index or zona-free hamster oocyte penetration assay

- Optimized SPA (SCI)
 - The methodological changes to the assay resulted in **polyspermy** for normal fertile males (with the lower limit: 5 spermatozoa/ovum)
 - improving sensitivity while maintaining the specificity and positive predictive value to predict IVF success (fertilization)
 - A positive score on this test was highly predictive of a positive outcome (fertilization) in IVF



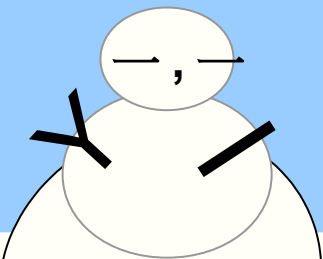
Tests of sperm DNA damage

- The causes of the DNA damage are largely unknown, although there is evidence to suggest that **genetic defects may underlie some sperm DNA damage.**
- The acridine orange staining test
- The sperm chromatin structural assay
- Terminal deoxynucleotidyl transferase-mediated nick end labelling
- The Comet assay



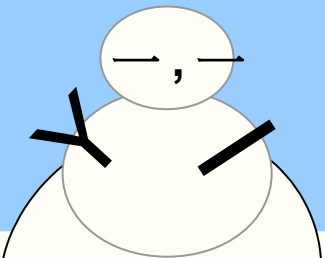
Tests of sperm DNA damage

- In general, significant DNA damage is rarely found in a proven fertile male, and the incidence of DNA damage is higher in infertile men.
- Recent studies suggest that sperm DNA damage levels can predict success using ART and they may also predict the likelihood of recurrent pregnancy loss.
 - Zini et al. suggested that sperm DNA damage may provide a useful biomarker of correction of detrimental fertility impairing conditions, such as varicocele.



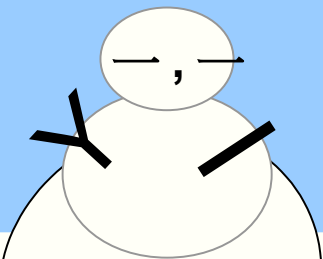
Tests of sperm DNA damage

- The incidence of DNA damage was lower in testicular spermatozoa compared with ejaculated spermatozoa.
 - ICSI with testicular spermatozoa might be advantageous to infertile men with high levels of DNA damage in their spermatozoa.



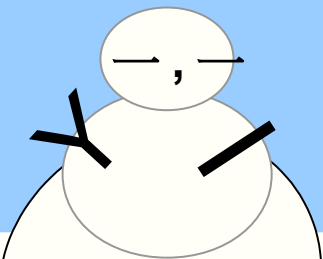
Tests of sperm DNA damage

- Additional highly controlled studies are required to define the functional significance of the DNA damage observed
 - the molecular basis for the damage and methods to prevent or ameliorate damage.
- DNA damage would not be detected in a routine semen analysis, making this test an important part of the evaluation of the infertile male.



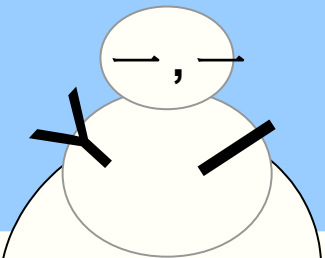
Assessment of reactive oxygen species

- Oxidative stress presents a major challenge in many aspects of medicine, pharmacology and cellular function.
 - The generation of free radicals is a normal by-product of cellular metabolism ex., oxygen ions and peroxides.
- Naturally occurring antioxidants are present to protect cells, but cellular damage can occur when the normal homeostatic mechanisms are unbalanced.
 - Reactive oxygen species (ROS) have been detected in the semen of some infertile men.



Assessment of reactive oxygen species

- McLeod, who reported that incubation of spermatozoa under conditions of high oxygen tension leads to immotility.
 - The addition of catalase, an antioxidant, preserved motility



Assessment of reactive oxygen species

- Neutrophil
 - the predominant leukocyte type in semen
 - a firm correlation between seminal WBC concentrations and ROS has been difficult to define
- Other sources of free radicals are iatrogenic, lifestyle and environmental factors, local and systemic infection, and autoimmune, inflammatory, testicular and chronic disease.

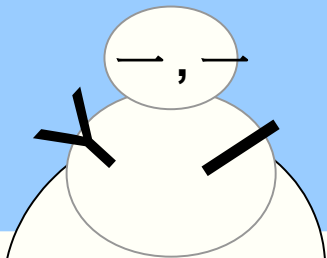


Assessment of reactive oxygen species

- Management:

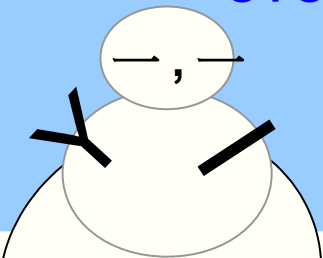
- Changes in lifestyle and exposures, supplementation with vitamins and antioxidants and treatment of infection and inflammation

- Improvement in pregnancy rates has not been as obvious



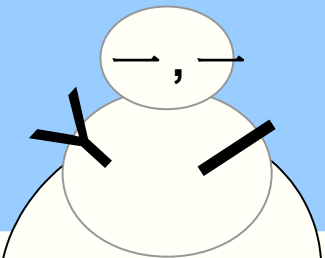
Assessment of reactive oxygen species

- Research in the area of oxidative stress and male infertility is clearly needed.
 - the efficacy of treatments in current clinical practice, such as administration of antioxidants to patients to prevent or ameliorate ROS generation.
 - With these treatments, together with lifestyle modifications that avoid toxins, improvement in sperm quality is generally expected; these treatments may even allow for natural conception for some couples.



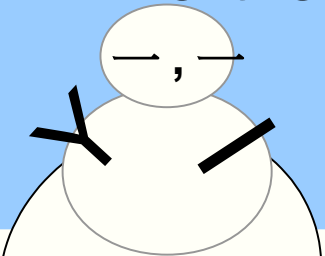
Chromosomal aneuploidy in sperm

- Delivery of the proper chromosome complement by the spermatozoon to the ovum is arguably the most important function of sperm.
- **Fluorescence in situ hybridization** provides a method to test for sperm chromosomal aneuploidy.



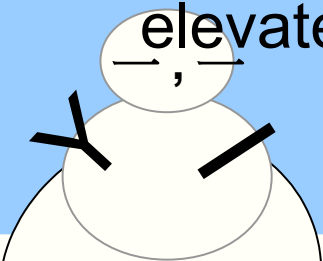
Chromosomal aneuploidy in sperm

- A spermatozoon that is disomic or nullisomic → Fertilization results in monosomic or trisomic embryos
 - the majority of which are incompatible with a viable birth
- The spermatozoa of infertile men exhibit a 10x higher incidence of chromosome aneuploidies (testing for chromosomes X, Y, 13, 18, 21— those compatible with a live birth) compared with fertile men.



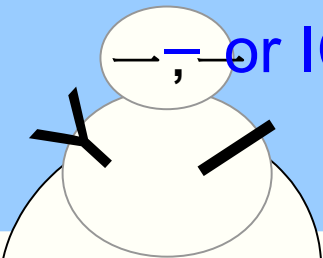
Chromosomal aneuploidy in sperm

- The increased incidence increases as the standard semen parameters worsen.
- Retrospective studies support the idea that an elevated rate of aneuploidy in spermatozoa is linked to the birth of a child (or conceptions) with aneuploidy.
- Prospective studies show that **men with elevated sperm aneuploidy are at increased risk of producing aneuploid embryos, IVF failure or aneuploid conceptuses.**
- Recurrent pregnancy loss is also associated with elevated sperm aneuploidy.



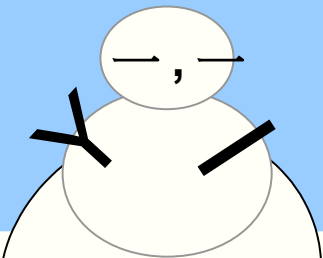
Chromosomal aneuploidy in sperm

- For couples with recurrent pregnancy loss, knowledge of increased sperm aneuploidy, together with genetic counseling, allows them to make informed reproductive decisions
 - remaining childless
 - using donor sperm
 - using preimplantation genetic diagnosis together with ICSI–IVF
 - or to continue their efforts to achieve a naturally conceived
 - or ICSI–IVF mediated pregnancy



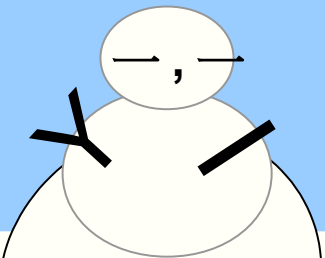
Final comments

- Why the evaluation of the infertile male requires advanced, specialized andrology assessment of parameters, such as genetics or sperm function, if all that is needed is a single spermatozoon to achieve a pregnancy with ICSI (the logic being that if a fertilization can occur, a healthy baby is likely to have been conceived)?
 - even healthy fertile couples may conceive a child with significant genetic or birth defects



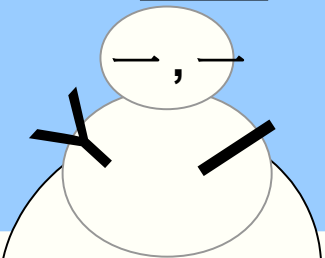
Final comments

- Some of the tests of sperm function described above can provide important information to infertile couples to aid in their reproductive decision making.
 - In some cases, sperm function testing may indicate that less expensive technologies may help a couple seeking to conceive a child; ICSI–IVF may not always be required.



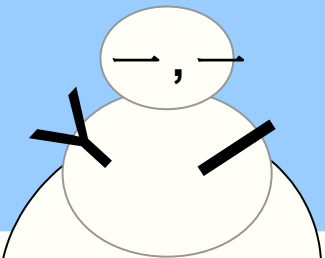
Final comments

- In other cases, such as **men who fail the hamster ICSI–SPA test**, donor spermatozoa or other options must be considered.
- The real strength of sperm function testing lies in its ability to identify men with normal semen parameters but who have functionally deficient spermatozoa that will fail to fertilize in routine IVF.



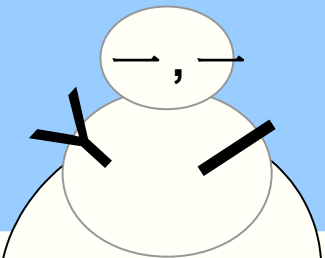
Final comments

- Our understanding of the genetic defects that underlie defects in sperm function, as well as the processes of spermatogenesis and fertilization, has improved.
 - Although diagnostic tests are not currently available for each gene, other tests, such as those developed to assess sperm aneuploidy, provide important information to couples planning to use ART.



Final comments

- The use of these tests to identify couples absolutely requiring ICSI– IVF to achieve a pregnancy, or even those who are likely to fail to achieve a pregnancy with ICSI–IVF, will allow physicians to provide a cost-efficient treatment plan for each infertile male factor couple.
 - Sperm function tests can provide increased knowledge of the causes of infertility



Final comments

- Ultimately, it is the couple's decision to seek to achieve a pregnancy; infertile couples, however, deserve the right to decide whether this is a realistic goal with a reasonable likelihood of success.
- Thus, sperm function testing, as well as other genetic and andrology tests now available, allow couples to make a more informed decision.

