Intracytoplasmic Injection with Suboptimal Spermatozoa

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Background

Since the report of the first birth with in vitro fertilization (IVF) in 1978, this procedure has been used extensively for the alleviation of human infertility [1]. However, because spermatozoa cannot fertilize in many cases of male factor indication, a number of supplementary techniques emerged to overcome this inability, and these are generally referred to as assisted fertilization, microsurgical fertilization, or simply gamete micromanipulation. The application of microscopic surgery to human gametes has allowed the achievement of fertilization in cases of severe oligo-astheno-terato-zoospermia and with dysfunctional spermatozoa. In addition, it has served as a powerful tool for a comprehensive understanding of the basic elements of oocyte maturation, fertilization, and early conceptus development. Gamete micromanipulation techniques now permit the diagnosis and at times even the correction of genetic anomalies, as well as optimization of embryo implantation chances in selected cases.

When sperm density, motility, and morphology are inadequate, various techniques have been proposed to bypass the zona pellucida. Zona drilling (ZD) [2] involved the creation of a circumscribed opening in the zona by acid Tyrode's solution delivered through a fine glass micropipette. It inevitably became clear that the use of an acidic medium had a deleterious effect on the one-celled egg—an effect not reported in cleavage-stage embryos using the "hatching" procedure. Alternative procedures were zona cracking in which the zona was breached mechanically with two fine glass hooks [3] and zona softening performed by a brief exposure to trypsin [4] or pronase. Partial zona dissection (PZD) [5] involved slicing of the zona with glass needles prior to exposure of the treated oocytes to spermatozoa. The above listed approaches carried a distinct risk of injury to the oocytes and aimed at producing an opening in the zona of proper size to facilitate penetration of spermatozoa in the perivitelline space and then fusion with the oolemma. Localized laser photoablation of the zona was even tested in this regard to produce a gap of precise dimensions within the zona, and this has resulted in a few offspring [6, 7]. However, not only did all these early procedures brought a modest and inconsistent fertilization, with PZD being the most used in that regard, but they were plagued by an unacceptably higher incidence of polyspermy. The mechanical insertion of spermatozoa directly into the perivitelline space—subzonal sperm injection (SUZI) [8]—was introduced as another option of overcoming inadequacies of sperm concentration and motility, and

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this proved to be more effective than ZD or PZD, particularly following prior induction of the acrosome reaction [9, 10]. However, SUZI also remained limited by the inability to overcome acrosomal abnormalities or dysfunction of the sperm–oolemma fusion process, and, ultimately, by disappointingly low fertilization rates.

Because ICSI involves insertion of a single selected spermatozoon directly into the oocyte, this bypasses all the preliminary steps of fertilization. The technique was initially attempted in lower organisms, such as the sea urchin [11] and then in mammalian oocytes [12] with the observation of a sperm nucleus decondensing after its microinjection into hamster eggs [13] with subsequent male pronucleus formation [14, 15]. This approach obviously caused oocyte injury and lysis [16], and in early studies only about 30 % of injected mouse eggs survived the procedure, even when supposedly fine micropipettes were used [17].

Because the gamete fusion step in ICSI fertilization is bypassed, male pronucleus development generally requires oocyte activation in most species tested and this can be granted by energetic suction of some cytoplasm immediately before or during sperm nucleus insertion [18]. The first live offspring using sperm injection were obtained in the rabbit following the transfer of the so inseminated oocytes into the oviduct of a pseudopregnant female [19], and soon after a single live birth was reported in the bovine [20]. Although applied to human gametes some years earlier [21], the first human pregnancies with ICSI occurred only in 1992 [22].

When Is ICSI Used?

The intracytoplasmic sperm injection (ICSI) procedure entails the deposition of a single spermatozoon directly into the cytoplasm of the oocyte, thus bypassing the ZP and the oolemma. The ability of ICSI to achieve higher fertilization and pregnancy rates regardless of sperm characteristics makes it the most powerful micromanipulation procedure yet with which to treat male factor infertility. However, no universal standards for patient selection have been defined for ICSI. The general consensus is that ICSI may be adopted when an extremely poor sperm sample is noted or following fertilization failure using in vitro insemination techniques.

Although oocytes that failed to fertilize with standard IVF techniques can be reinseminated by ICSI, this introduces a risk of fertilizing aged eggs [23]. In our own limited experience, six of eight pregnancies established by micromanipulation of such oocytes miscarried, and cytogenetic studies performed on conceptuses provided evidence of chromosomal abnormalities. Thus, notwithstanding reports of normal pregnancies [24, 25] the reinsemination of unfertilized oocytes is currently not advisable for routine clinical application.

When initial sperm concentration in the ejaculate is $<5 \times 10^6$/ml, the likelihood of fertilization with standard IVF is significantly reduced [26], and therefore such couples should be considered unsuitable for this technique, particularly where <1 % normal forms are observed. However, fertilization of mature oocytes may still fail to occur in the presence of normal sperm [27] because of the hardening of the zona pellucida [28], or when oocytes reveal ooplasmic inclusions [29, 30]. Abnormalities of the zona pellucida prevent sperm fusion with the oolemma [31] thus justifying sperm injection. In most instances, however, failure of fertilization is due to coexisting sperm abnormalities presenting ICSI as the only treatment option [32].

Early experience showed that isolated nuclei of testicular and epididymal hamster spermatozoa decondensed soon after injection into mature hamster oocytes, and formed pronuclei in activated eggs [33]. Although in vitro fertilization of human oocytes was accomplished with epididymal spermatozoa in men with obstructive azoospermia [34, 35], only with the advent of ICSI it was possible to obtain consistent fertilization with each gamete source [36–38]. Testicular biopsy was employed to retrieve sperm cells from men who had a scarred epididymis and therefore, no chance of retrieval through that route [39, 40]. However, the therapeutic possibilities of ICSI go even further since immotile testicular spermatozoa and supposedly even spermatids have been successfully used [41].

Some men produce only round-headed spermatozoa which have no acrosome and can neither bind to nor penetrate zona-free hamster oocytes [42, 43]. However, ICSI has enabled even such acrosomeless spermatozoa to establish pregnancies [44–48]. Moreover, ICSI's dependability has broadened its initial use from a technique capable of overriding the dysfunctionality of spermatozoa to one that may partly compensate for problems with the egg. Indeed, ICSI has allowed successful fertilization when only a few and/or abnormal oocytes were available [49]. Stripping cumulus cells from the oocytes allows a direct assessment of maturation, thus offering a woman with a limited number of oocytes a much greater chance of successful fertilization. In fact, the availability of ICSI has been instrumental in some European countries that include Italy and Germany in circumventing restrictive legislation that limits the number of oocytes inseminated or embryos to be replaced [50–52].

ICSI made it possible to have more consistent fertilization when injecting cryopreserved oocytes [53]—overcoming the problem that freezing can lead to premature exocytosis of cortical granules, resulting in zona hardening and inhibition of natural sperm penetration [54–57]. ICSI is also the preferred conception method during the application of preimplantation genetic diagnosis (PGD) because it avoids DNA contamination from additional sperm adhering to the zona and it enhances the number of fertilizable oocytes and ultimately embryos available for screening [58].

ICSI also has an impact in the arena of HIV infection in serodiscordant couples. Three-quarters of individuals infected by HIV or HCV are in their reproductive years. Male-to-female transmission of HIV is estimated to be only 1 per 1,000 acts of unprotected intercourse [59] and even higher in HCV infected patients [60]. Moreover, because of antiretroviral therapies, the course of HIV-1 infection has shifted from a lethal acquired immunodeficiency syndrome to a chronic manageable disease. In such cases, intrauterine insemination (IUI) with spermatozoa processed by double gradient centrifugation followed by swim up has been the suggested method of treating couples with an HIV-1-infected male partner [61]. However, the use of ICSI has been proposed by several groups because of its negligible oocyte exposure to semen, thereby reducing the risk of viral transmission [62, 63]. Advantages of ICSI over IUI also include the considerably higher success rate [62], requiring fewer attempts to achieve pregnancy while reducing viral exposure [64]. Fortunately, so far, no seroconversions have been reported following ART treatments including IUIs [65, 66].

Finally, because only a single spermatozoon is needed for each egg, ICSI has allowed treatment of men who are virtually azoospermic (also defined as cryptozoospermic) [67]. Such cases of spermatogenic arrest have necessarily involved the injection of immature spermatozoa or even spermatogonia [40, 41, 68, 69]. Nonetheless, where fertilization occurs in ICSI cases, conception is accomplished with an embryo implantation that follows a success pattern, at least in our experience, comparable to that seen with standard in vitro insemination.

Clinical Outcome

In the last 19 years at Cornell, we have performed a total of 34,425 ART cycles. Of those, 31.7 % (10,898) included the standard in vitro insemination cycles; the average maternal age was 37.6 ± 4 years and paternal age of 39.6 ± 6 years that resulted in a fertilization rate of 60.5 % and a clinical pregnancy rate of 37.6. In vitro insemination was generally performed in patients with ideal semen parameters, while ICSI has been used to treat couples with suboptimal spermatozoa, a history of poor fertilization, and/or limited numbers of oocytes.

ICSI was performed in 21,302 cycles with ejaculated spermatozoa with a mean maternal age of 36.9 ± 5 years and paternal age of 40.8 ± 8 years. In our patient population 18,757 of our men had at least one abnormal semen parameter according to the WHO 2010 criteria. In these suboptimal sperm cohort, of the 175,833 MII oocyte injected, 5.1 % lysed and those that survived yielded 79.2 % (132,183/166,796)

Table 2.1 Fertilization and pregnancy rates according to semen origin

	Spermatozoa	
No. of	Ejaculated	Surgically retrieved
Maternal age (M ± SD years)	36.9 ± 5[a]	35.1 ± 5[a]
Cycles	21,302	2,225
Fertilization (%)	132,183/166,796 (79.2)[a]	12,922/20,779 (62.2)[a]
Clinical pregnancies (%)	8,404 (39.5)[b]	993 (44.6)[b]

[a]χ^2, 2 × 2, 1 *df*, effect of spermatozoal source on fertilization rate, $P = 0.0001$
[b]χ^2, 2 × 2, 1 *df*, effect of spermatozoal source on clinical pregnancy rate, $P = 0.0001$

Table 2.2 Spermatozoal parameters and intracytoplasmic sperm injection (ICSI) outcome according to retrieval sites and specimen condition

	Spermatozoa			
	Epididymal		Testicular	
No. of	Fresh	Frozen/thawed	Fresh	Frozen/thawed
Cycles	342	624	917	342
Density (10^6/ml ± SD)	45.8 ± 47	26.6 ± 32	0.4 ± 2	0.2 ± 0.7
Motility (% ± SD)	19.0 ± 17[a]	4.1 ± 8[a]	3.1 ± 7	1.2 ± 4
Morphology (% ± SD)	1.7 ± 2.3	1.2 ± 2	0	0
Fertilization (%)	2,515/3,473 (72.4)	4,104/5,779 (71.0)	4,894/8,568 (57.1)[b]	1,406/2,959 (47.6)[b]

[a]Student's *t*-test, two independent samples, effect of epididymal cryopreservation on sperm motility, $P < 0.0001$
[b]χ^2, 2 × 2, 1 *df*, effect of testicular cryopreservation on fertilization rates, $P = 0.0001$

zygotes. Of the oocytes that abnormally fertilized, 4,170 (2.5 %) displayed 1PN and 5,838 (3.5 %) were 3PN. The clinical pregnancy rate, as detected by the presence of at least one fetal heartbeat, was 39.5 % (Table 2.1).

When more immature forms of spermatozoa were utilized, for example those surgically retrieved, the fertilization rate of 62.5 % and although satisfactory was lower than that achieved with ejaculated spermatozoa ($P = 0.0001$) (Table 2.1). In contrast, the clinical pregnancy rate appeared lower in the ejaculated group in comparison to the surgically retrieved spermatozoa; this difference may be attributed to the younger maternal age in the latter cohort.

In situations where no spermatozoa were found in the ejaculate after two semen analyses, patients opted to undergo epididymal or testicular sperm retrieval. In 2,225 cycles with surgically retrieved spermatozoa, the mean maternal age was 35.1 ± 5 years. A total of 966 cycles were performed with epididymal specimens and 1,259 cycles with testicular samples. When looking at men with obstructive azoospermia that used spermatozoa retrieved from the epididymis, those diagnosed with congenital absence of the vas ($n = 524$) had superior fertilization (72.1 % vs. 70.9 %; $P = 0.0001$) as well as higher clinical pregnancies (54.0 % vs. 46.8 %; $P = 0.03$) in comparison to those that had an acquired vas obstruction ($n = 442$). In cycles that used testicular sampling, we divided them according to their etiology as being obstructive ($n = 228$) or nonobstructive ($n = 1{,}031$). In these cases, the fertilization rate was superior in the obstructive cohort when compared to the nonobstructive group (64.5 % vs. 52.7 %; $P = 0.0001$) but resulting in comparable clinical pregnancies (45.2 % vs. 38.8 %).

When the fertilization and pregnancy characteristics were analyzed according to whether the sample was cryopreserved, we observed that after cryopreservation epididymal samples had lower motility parameters ($P < 0.0001$; Table 2.2) as well as pregnancy outcome ($P = 0.0001$; Fig. 2.1), though without affecting fertilization rate (Table 2.2). When testicular samples were used for ICSI, the situation was reversed with zygote

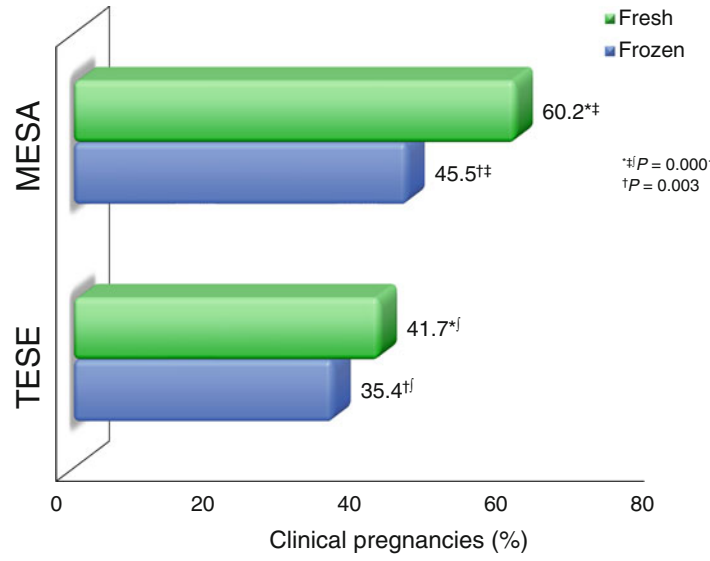

Fig. 2.1 Clinical pregnancy according to type of surgical sampling and specimen condition

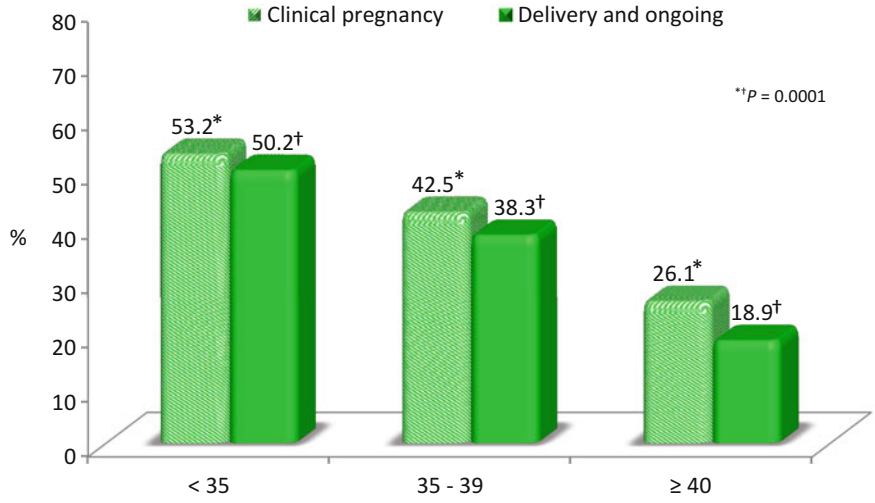

Fig. 2.2 Clinical outcome per oocyte retrieval grouped according to maternal age. Clinical pregnancy is considered as the presence of at least one fetal heartbeat

formation being higher in the fresh specimens ($P=0.03$) as well as the ability of the embryo to implant ($P=0.0001$; Table 2.2; Fig. 2.1).

When 21,028 ICSI cycles (after exclusion of the donor egg cycles) were plotted as a function of increasing maternal age, there was a progressive decrease in pregnancy ($P=0.0001$; Fig. 2.2) and consequently in delivery rates ($P=0.0001$). As predicted, there was a higher incidence of miscarriages, therapeutic abortions, and overall pregnancy losses as a function of the age of the female partner ($P=0.0001$), pregnancy wastage being 2.6 times greater in women ≥40 years compared to those of <35 years.

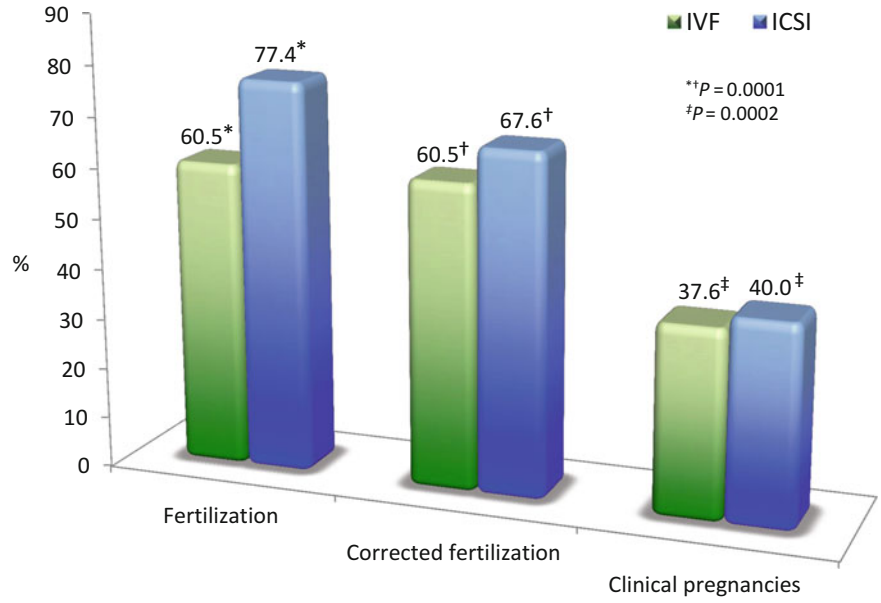

Fig. 2.3 Fertilization and clinical pregnancy reported at Cornell following standard in vitro insemination and ICSI. To better compare fertilization success between the two insemination methods, we have corrected fertilization with ICSI using the total number of oocytes retrieved as the denominator

A total of 7,422 ICSI patients delivered 9,150 babies comprising 4,606 males and 4,521 females (with 23 unknown genders). A total of 3.6 % (330) exhibited congenital abnormalities at birth, of which 174 (1.9 %) were major and 156 (1.7 %) were minor. IVF children ($n=5,183$) had a comparable overall malformation rate (104 major and 83 minor).

To evaluate differences in performance between insemination methods, we compared embryological outcomes and clinical pregnancy rates between standard in vitro insemination and ICSI. While it appeared that fertilization was lower in IVF than with ICSI ($P=0.0001$; Fig. 2.3), after correcting for all retrieved oocytes and not for metaphase II injected, ICSI still yielded more oocytes fertilized (60.5 % vs. 67.6 %; $P=0.0001$). Furthermore, the ability to generate term pregnancies was also higher with the ICSI cohort ($P=0.0002$). However, as in all fields of reproductive medicine, the limiting factor remains to be maternal age (Fig. 2.2), as evidenced by an inverse relationship between delivery rate and female age [70].

The Quest for the Ideal Spermatozoon

While ICSI has been the gold standard for most IVF centers for more than 20 years with no proven significant or attributable side effects, some researchers still question the possible deleterious effects of a technique that bypasses the natural gamete selection processes typical of in vivo reproduction. Towards that goal, several methods have been introduced that expound upon the procedures of ICSI with additional protocols aimed at finding the optimal spermatozoon to inseminate an oocyte.

It is difficult to select spermatozoa in terms of morphology while they are in motion and without the use of stains. However, selection of normally shaped spermatozoa can be accomplished to a certain extent by observing their shape, light refraction, and motion patterns while screening them in a viscous medium.

Initial preparation methods were based on a simple separation of spermatozoa from the seminal fluid referred to as "washing technique" [71].

Subsequently, the spermatozoa were selected according to sperm motility by the migration or swim up method [72]. Later methods were mainly based on sperm density (mass/volume) in order to select viable and motile spermatozoa with normal morphology. These methods have been mainly referred to as density gradient centrifugation (DGC) techniques that are commonly used for sperm processing in different centers [73]. Alternative methods infrequently used are mostly based on forcing spermatozoa to swim through a variety of artificially created hurdle paths such as glass wool filtration [74], Tea-Jondet Tube [75], and Wang's Tube [76] and Sephadex [77], just to name a few.

The majority of these techniques not only recover viable spermatozoa with normal morphology, but it is believed that they can also, to different degrees, recover mature spermatozoa with intact chromatin and DNA [78]. Because spermatozoa contribute to approximately half of the genome of the next generation, selection of spermatozoa with intact chromatin for the ICSI procedure should become mandatory. Sperm DNA integrity is currently assessed by destructive methods such as TUNEL, COMET, sperm chromatin dispersion (SCD) test, or by a sperm chromatin structural assay (SCSA). However, all of these require fixation and so loss of the sperm cell being evaluated [79]. As assessment of chromatin integrity while observing sperm viability is not plausible, researchers have tried to select spermatozoa mainly based on their surface characteristics and attempted to establish a relationship with sperm genetics and epigenetic traits such as DNA integrity or ploidy.

Recently, our attention has been directed towards the unicellular approach for studying the male gamete aiming at reading its chromosomal constitution [80–82] or its chromatinic integrity [83–85]. Suspending spermatozoa in viscous medium allows the observation of their 3D kinetic patterns [86, 87] and to evaluate their morphological characteristics at high magnification [88, 89]. While new insights are being established on surface markers of the spermatozoon [90–92], a clearer understanding of the conformational chromatin structure characterized by the two forms of DNA present (protamine and histone bound) and the recent recognition of small noncoding RNA [93–95] will guide the treatment of infertility through the next generation.

It has been postulated that fertile men with normal semen parameters almost uniformly have low levels of DNA breakage, whereas infertile men with compromised semen parameters presumably present with nicks and breaks in their sperm chromatin. However, in these men spermatozoa with compromised DNA integrity, measured by the most popular methods, do not seem to correlate with sperm concentration and morphology [96, 97]. In a systematic observation carried out in our laboratory, instead we have reported a strong inverse correlation between DNA fragmentation (measured by SCSA and TUNEL) and kinetic characteristics [98]—as motility decreases, there was an increase in DNA fragmentation. Perhaps this may explain why there is a lack of predictability between DNA integrity and pregnancy outcome with ICSI inseminations [99] because of the fact that only motile spermatozoa are utilized for injection regardless of their number.

The pledge for the ideal spermatozoon has been perceived as a surface scrutiny under high magnification of the individual sperm cell dubbed "motile sperm organellar morphology examination" (MSOME) [88]. This hinted to "intracytoplasmic morphologically selected sperm injection" (IMSI) that claimed to yield superior clinical outcomes than conventional ICSI [88, 89]. IMSI promised higher fertilization, implantation, clinical pregnancy rates along with lower pregnancy losses and healthy offspring in a series of studies [100–103]. Higher magnification screening of sperm surface irregularities, however, did not prove the asserted amelioration of clinical outcome in independent investigations. This has been true for male factor couples, at first or repeated ART attempts [87]. Moreover, light microscopic observations of surface sperm head irregularities or vacuoles are almost ubiquitous once higher level examination, i.e., by transmission electron microscopy (TEM), scanning electron microscopy (SEM), and confocal microscopy suggesting a paraphysiologic nature of these entities [87, 104–106]. In fact, these vacuole-like structures, or as more appropriately described craters, appear

in over 90 % of spermatozoa from fertile donors with normal semen parameters [107, 108]. The whole concept of IMSI may possibly be suited for cases where millions of morphologically normal spermatozoa are available for selection, but in fact cannot practically be employed in severe oligozoospermic cases where cryptozoospermia and nonobstructive azoospermia only yield scarce viable cells.

A connection between a specific phenotype and the intrinsic chromosomal/chromatinic integrity of the spermatozoa has also been attempted through the hyaluronic acid binding characteristics appearing on the surface of the mature sperm cells [90–92]. This biochemical marker was verisimilarly used to identify the most viable mature spermatozoa with intact DNA, euploid, and restricted amount of histones, and achieve embryo developmental competence [90–92] to be used for ICSI. However, this concept is somewhat contradicted by the observation that immature spermatozoa, such as those retrieved from epididymis and testes, are capable of generating high fertilization and pregnancy rates in a comparable manner to their ejaculated counterparts (see Table 2.2; Fig. 2.1). PICSI, or "Physiologic ICSI," makes use of hyaluronic acid (HA), a substance naturally present in the human body [109]. HA can be found in the cumulus oophorus around the oocyte and represents a barrier to the immature gametes by only relenting to "mature" spermatozoa. These putatively ready spermatozoa that have undergone the complete process of plasma membrane remodeling, cytoplasmic extrusion, and nuclear maturity will have a significantly higher number of HA receptors and binding sites. Two methods have been proposed on how to perform PICSI. The first is an ICSI dish coated with microdots of hyaluronic acid hydrogel that allow HA-bound spermatozoa to be recovered using a standard ICSI injection pipette [92]. The other method is represented by a viscous medium composed partially of HA [109] that also fully replaces PVP. Some studies have shown that spermatozoa capable of HA binding have lower DNA fragmentation than simple post-swim-up spermatozoa. In addition, nucleus normalcy rate (according to MSOME criteria) has been shown to be higher in spermatozoa bound to HA as compared to spermatozoa in PVP [109]. However, PICSI correlations to pregnancy or delivery rates or malformation incidence have been inconsistent. In a systematic observation performed in our laboratory we carried out the selection of spermatozoa that exhibit HA binding sites on which we assessed chromosomal status and chromatinic competence. HA-bound and HA-unbound sperm cells were individually picked up by an ICSI pipette and assessed by Diff-Quik™, Aniline Blue, SCD, TUNEL, and FISH (Fig. 2.4). Male gamete genetic and epigenetic characteristics according to the expression of HA-binding sites are illustrated in Fig. 2.5. Surprisingly, the arrays of assays were within the expected limits for each individual test thresholds and across the HA expression characteristics. Although there were some improvements in the outcome of the tested parameters of the spermatozoa selected upon their motility characteristics, HA selection technique did not seem to add any further advantage [110]. Ultimately, PICSI is still impacted by the same major drawbacks as IMSI, represented by cases where extremely few sperm cells are present and therefore, rendering unworkable the putative selection.

ICSI with Unselected Spermatozoa

ICSI was exclusively developed to assist those with severe male factor infertility and this can include a wide variety of spermatogenic defects as often seen in cryptozoospermic and azoospermic men. In the latter case, the solution is to extract spermatozoa directly from the seminiferous tubules. In the micro-testicular sperm extraction (micro-TESE) procedure performed at our facility, the larger opaque seminiferous tubule is selected for excision [111]. This novel approach has greatly enhanced the chances of identifying spermatozoa in comparison to a random testicular sperm extraction while limiting scarring. Even with this targeted sampling approach, testicular surgery carries surgical and anesthesiological risks and those factors need to be carefully evaluated and discussed with patients particularly when seldom ejaculated spermatozoa are present. Independently of the technique used for the

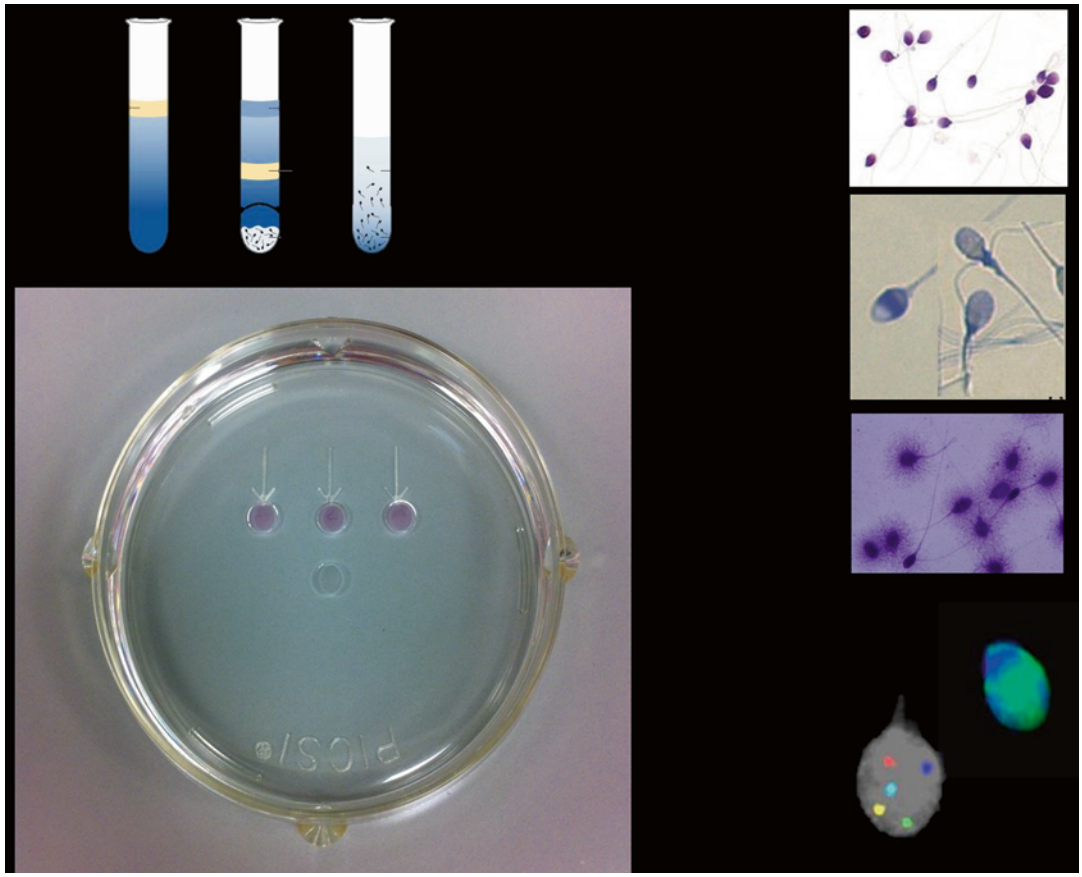

Fig. 2.4 Genetic and epigenetic assessment of spermatozoa after hyaluronan selection

retrieval of spermatozoa in men with compromised spermatogenesis, the minute amount of sperm cells available requires an exhaustive search for the much needed gamete to inseminate all oocytes. At times these extreme searches do not yield enough spermatozoa and often cryopreservation of the surplus oocytes need to be contemplated.

To provide an idea of the results obtained following the gargantuous effort during an extended sperm quest carried out on an inverted microscope while searching in droplets under oil by several embryologists, we grouped these cases according to time, 30 min–1 h, 1–2 h, 2–3 h, and >3 h, and compared to a control requiring less than 30 min [112]. Embryo development and implantation were recorded for the different sperm quest times. Independently of the source whether ejaculated or surgically retrieved, an exhaustive search for spermatozoa is needed to retrieve all spermatozoa for injection. In spite of the increasing search time and the extremely limited number of sperm cells identified, when oocytes were finally injected, fertilization did not dramatically differ in function of time and/or sperm source (control 58.9 % TESE vs. 75.6 % Ejac, $P<0.0001$; 30 min–1 h was 55.6 % TESE vs. 56.2 % Ejac; 1–2 h was 50.5 % TESE vs. 52.5 % Ejac; 2–3 h was 32.7 % TESE vs. 33.9 % Ejac; and >3 h was 27.8 % TESE vs. 33.3 % Ejac). Similarly, for both gamete provenance clinical pregnancies maintained a satisfactory clinical profile in spite of the increasing time spent to identify the spermatozoa (30 min–1 h was 51.6 % TESE vs. 35.4 % Ejac; 1–2 h was 44.6 % TESE vs. 57.1 % Ejac; 2–3 h was 34.4 % TESE vs. 0 % Ejac; and >3 h was 26.7 TESE vs. 100 % Ejac) [113].

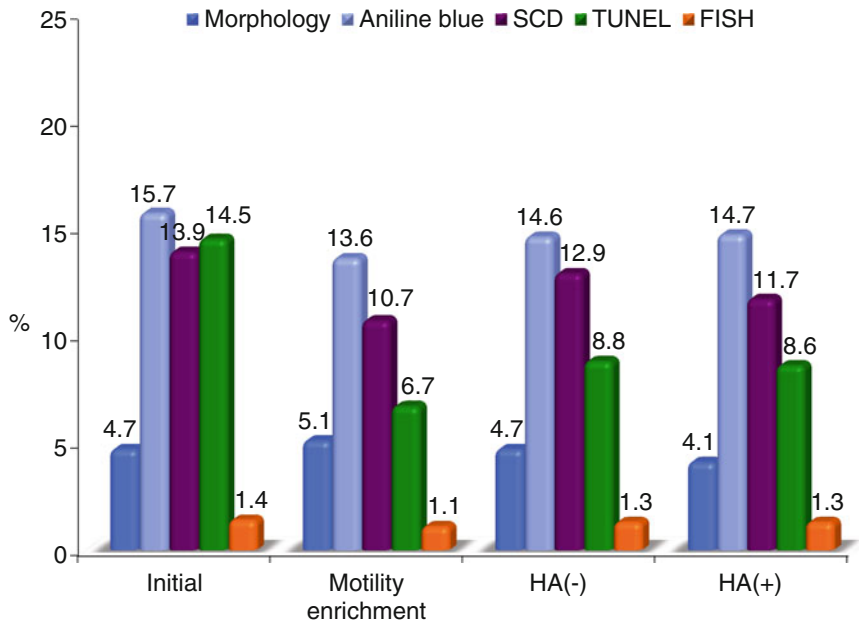

Fig. 2.5 Assessment of spermatozoa in raw semen, after sperm selection by density gradient, and after microtool pick-up of spermatozoa that were either bound to hyaluronan (HA+) or unbound (HA−)

Safety and Conclusions

Since the early establishment of in vitro insemination it became clear that a large portion of couples would not be capable of achieving fertilization. We have been involved since the early efforts in devising methods for assisted fertilization to allow men with subfertile spermatozoa to generate conceptuses once in vitro insemination of their partner oocytes failed in previous attempts. It quickly became evident that among the different approaches the direct injection of a spermatozoon would be the most effective way to solve male gamete dysfunction. Now ICSI is generously applied worldwide for a variety of indications and not exclusively for male factor infertility. ICSI has been shown to be the procedure of choice when spermatozoa, such as in azoospermic men, are directly retrieved from the epididymis and the testis. In fact in these men, as long as a viable spermatozoon is isolated, there is a chance of generating a conceptus. The fertilization achieved with surgically retrieved specimens matches those seen with optimal ejaculated gametes and similarly, embryo development is uncompromised.

Concerns raised by this invasive procedure where a gamete is arbitrarily selected have proved to be mainly unfounded as the health and developmental potential of offspring born from ICSI are comparable to those born after standard in vitro insemination. The real concerns erupt from the fact that infertile men carry a higher incidence of chromosomal defects and, particularly in azoospermic men, even meaningful microdeletion(s) on a gonosome may be present. Likewise, azoospermia itself is associated with a higher incidence of aneuploidy in the germ cells due to meiotic errors and with possible increase in autosomal/gonosomal disomies.

Notwithstanding the large number of babies born following ICSI worldwide, concerns still exist as to whether the use of suboptimal spermatozoa can result in genomic or phenotypic abnormalities in the progeny [114]. In one of the earlier studies on the evolution of pregnancies after ICSI, it was observed that the rate of malformation was

2.6 % after ICSI [115]. An extension of the Cornell series which included a total of 14,333 ART children examined found that the incidence of overall malformation was comparable between the IVF and ICSI [70]. Evidence regarding the outcome of singletons born at term following ART is generally reassuring [116]. The increased risk of perinatal morbidity and mortality associated with singleton births has been linked to the infertility of the couple rather than the ART techniques used [117].

The specific concerns in regard to ICSI, whether real or theoretical [118–121], involve the insemination method, the use of spermatozoa with genetic or structural defects, and the possible introduction of foreign genes.

In summary, the most palpable factor that can lead to adverse outcomes in offspring conceived by IVF or ICSI is the high and higher gestational order. The occurrence of this phenomenon has induced the consideration of single embryo transfer policies to address this considerably. Small for gestational age and prematurity confirmed in the ART population also appears to find an explanation in the higher order of embryos transferred and therefore implanted. Once ART reigns in the incidence of multiple gestations, the health of ART offspring seems comparable to those spontaneously conceived even considering the older age of the female partners. Although perinatal outcomes such as prematurity, low birth weight, perinatal mortality, and increased incidence of malformations that have been observed with ART techniques, it is clear that the main culprit is related to infertility itself [70]. Overall, studies of children ranging from newborn to 14 years of age [122–129] have been reassuring in terms of perinatal outcome, IQ, and physical development [116]. Further follow-up on ICSI teenagers into adulthood should be continued to better understand the reproductive capacity of these youngsters.

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