

Update on *In Vitro* Fertilization Approaches

a report by

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It is questionable whether anybody foresaw the remarkable potential of *in vitro* fertilization (IVF) when Edwards and Steptoe reported the first IVF pregnancy (an ectopic pregnancy),¹ or even after Louise Brown, the first IVF baby, was born on July 25, 1978.² Thirty years and an estimated three million IVF births later, IVF has become routine and, indeed, has replaced many, if not most, more traditional treatment approaches to infertility.

Remarkably, at least in the US, this success was achieved outside of traditional federal research-funding channels, as neither Democratic nor Republican administrations have ever supported IVF-related research. Now representing approximately 1% of all US births, the country's IVF children bear witness that avoidance of federal grant support and reliance on market forces may represent a surprisingly efficient way to rapidly advance medical progress.³

The dramatic improvements in IVF outcomes observed over 30 years in the US were driven by market forces, at least in part. For a long time considered experimental and later elective (the obviously incorrect assumption being that infertility does not represent a disease state), in contrast to most developed nations, IVF in the US represents a mostly uncovered medical insurance benefit.⁴ Consequently, patients had no choice but to become educated consumers in search of the best returns for (quite considerable) investments in IVF costs. One of the obvious consequences was an outcome-driven IVF market, in which programs with better results (i.e. pregnancy rates) flourished.³

Therefore, it is not surprising that IVF pregnancy rates in the US are far higher than in Europe,^{5,6} where IVF is offered far more generously under state-sponsored universal health plans.⁷ However, government sponsorship also leads to government interventions, and the practice of IVF in Europe is often characterized by considerable regulation and/or legislation, with often quite detrimental effects on pregnancy outcomes.^{8,9}

Evolving outcome differences between North America (Canadian outcomes are closer to US results)¹⁰ and Europe have recently finally attracted attention, and are now subject to increasing scrutiny. However, they are reflective of the more basic question of whether continuous improvements in IVF outcomes, as witnessed over the last 30 years, are sustainable under current practices.

Continuous progress in IVF seems not only possible but, indeed, likely if uncontrolled systemic interventions can be avoided. Such systemic interventions refer not only to government regulations, but to any kind of systemic effort to modify the practice of IVF without appropriate consideration of outcomes. As IVF has matured and patients expect reasonable pregnancy rates, there simply is no longer a place for untested, grandiose intuitive

concepts in modern IVF. Recent, rather adverse, experiences with pre-implantation genetic screening (PGS) and single-embryo transfer (SET) have amply demonstrated this fact. Continued improvements in IVF will increasingly depend on better individualization of care and avoidance of universal protocols. In the following sections of this article, we will attempt to illustrate this concept further.

Treating Ovarian Rather than Chronological Age

Individualization of care starts with diagnosis. Nothing is more important than accurate diagnosis, which, of course, leads to appropriate treatment and *vice versa*. This is the primary reason why we object to the engrained utilization of so-called unexplained infertility (UI) as the diagnosis in approximately one-third of infertility patients.¹¹

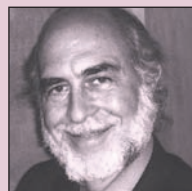
One of the most frequently overlooked infertility diagnoses, often mistakenly considered to be UI, is premature ovarian senescence, and especially the milder forms of this primary ovarian insufficiency (POI), which was recently well reviewed by Nelson in *US Obstetrics and Gynecology*.¹² In



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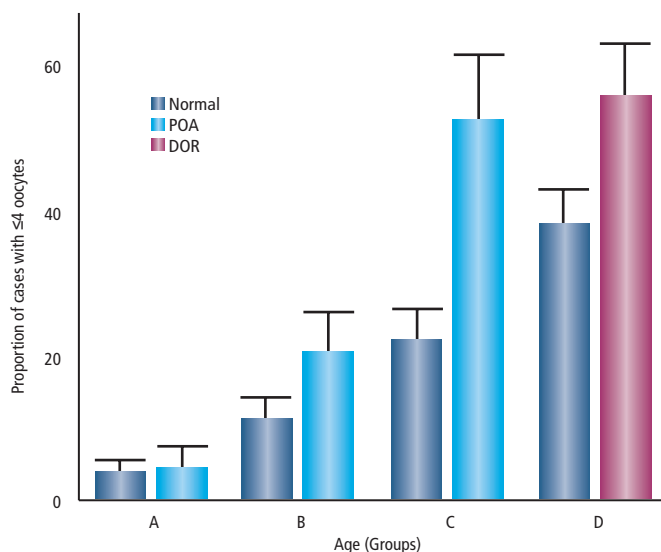
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Table 1: Age-specific Normal Upper Cut-off Levels for Baseline Follicle-stimulating Hormone*

Patient Age (Years)	Upper Limit of Baseline FSH (mIU/ml)
≤32	6.9
33–37	7.8
38–40	8.3
≥41	8.4

Represents 95% confidence interval of baseline follicle-stimulating hormone (FSH) levels of infertility patients in respective age groups at the fertility center of the authors; therefore, levels in a fertile population can be expected to be even lower. * Modified from Barad et al. with permission.¹¹

Figure 1: Prevalence of Ovarian Resistance at Various Ages in Premature Ovarian Aging and Normal Patients*



DOR = diminished ovarian reserve. The figure demonstrates the prevalence of ovarian resistance (OR), defined as retrieval of less than four oocytes, in women of various age groups. As can be seen, in each age group a definition of premature ovarian aging (POA) (baseline follicle-stimulating hormone > age-specific cut-off) defined a group of patients at significantly increased risk towards OR. * Modified from Barad et al. with permission.¹³

its most severe form and disease end-stage (premature ovarian failure [POF]), the condition will be obvious and easily diagnosed. However, at milder and more subtle stages a diagnosis will be more difficult, especially if the patient is young and ovarian compromise (in contrast to older women above 40 years of age) is unsuspected.

Milder degrees of POI have been called occult if the only hint of diminished ovarian reserve is resistance to ovarian stimulation with gonadotropins, biochemical if baseline follicle-stimulating hormone (FSH) levels are elevated, and overt if menstrual patterns have become irregular.¹² Since all three of these findings can occur in parallel, we have instead coined the term premature ovarian aging (POA) to describe women under 40 years of age who demonstrate any of the signs of POI.¹³ While POA can, of course, carry over into the 40s, a diagnosis at such an advanced age becomes more difficult because women over 40 years of age almost uniformly demonstrate evidence of diminishing ovarian reserve. The most logical end-point for a diagnosis of POA would probably be 37.5 years of age, commensurate with approximately 25,000 remaining follicles, when the physiological decline in female fertility is believed to accelerate.¹⁴

The objective definition of so-called ovarian resistance (OR) is difficult because it requires uniformity of ovarian stimulation and of outcome, i.e. oocyte yield.

Similarly, a definition of increasing menstrual irregularity can be difficult because menstrual patterns are often variable in women. Therefore, among the milder forms of POI, biochemical tests of ovarian reserve are clearly the most suitable criteria to reach an accurate diagnosis. Among these, baseline FSH has been the most widely utilized. Unfortunately, however, FSH fluctuates to a significant degree, is influenced by other hormones (such as the negative feedback with estradiol), and, probably most importantly, naturally increases with advancing female age.¹³

The latter point suggests that the current utilization of universal FSH levels for women of all ages does not make sense. Indeed, when we investigated age-specific FSH cut-off levels (defined as the 95% confidence interval [CI] of age-specific FSH levels), we demonstrated that abnormal FSH levels in practically all age groups started considerably below the generally used cut-off levels of 10–12mIU/ml (see Table 1). Moreover, in all age groups age-specific FSH levels were able to differentiate between women with and without OR (see Figure 1).¹³

This observation strongly supports the concept that an accurate assessment of ovarian reserve cannot be based on universal cut-off levels of normal and, instead, should rely on age-specific levels. When we performed this for our center's infertility population, we were surprised to find that approximately half of all women under 35 years of age demonstrated elevated age-specific FSH levels, thus qualifying for a diagnosis of POA.¹³ Who then can still be surprised about the high prevalence of the pseudo-diagnosis of UI, because without utilization of age-specific FSH levels even competent fertility experts will have difficulties recognizing milder forms of diminished ovarian reserve?

In place of and/or in combination with FSH, the assessment of ovarian reserve is increasingly also utilizing the evaluation of anti-Müllerian hormone (AMH). In principle, AMH correlates well with FSH,¹⁵ but has superior predictive value in terms of oocyte yields and pregnancy rates in IVF.¹⁶ Here too, largely universal uniform levels are still utilized for all ages. Analogous to FSH, we are in the process of establishing age-specific AMH levels, expecting them, in combination with age-specific FSH, to improve the assessment of ovarian reserve, thus permitting improved accuracy in early diagnosis of POA.

An early diagnosis of POA is of crucial importance because it changes all existing treatment paradigms. Since POI invariably leads to early menopause,¹⁴ younger women who, with normal and age-appropriate ovarian function, seemingly have all the time in the world to conceive, suddenly may find themselves under much more time pressure. However, how early functional menopause can be expected may be difficult to predict and advise; therefore, worst-case scenarios have to be assumed. Young women suspected of POA, previously not yet ready/willing to conceive, now have the options of fertility preservation via oocyte and/or embryo cryopreservation.¹⁷

Even more importantly, those ready to conceive suddenly face a radically different treatment paradigm since their ovaries behave 'older' than their chronological age suggests. In other words, while infertility treatments (including IVF) are usually age-specific, POA patients should no longer be treated in accordance with chronological age, but should be based on ovarian age. This represents a radical shift in treatment paradigms because older women not only face more urgency, but also are usually entirely differently

stimulated. Therefore, everything starts with accurate diagnosis, and from there carries over into appropriately designed protocols. When all of this happens, usually quite dismal pregnancy rates in POA patients will still be surprisingly high.¹⁸

An Increasingly Older Patient Pool

As already previously noted, advanced female age is associated with progressively diminishing ovarian reserve. Therefore, the older the female, the poorer her IVF prognosis.¹⁹ In the US, women over 40 years of age represent the most rapidly expanding age group giving birth.^{20,21} Therefore, it should not be surprising that the infertility population in treatment has been aging.^{22,23}

However, surprisingly, a strong argument can be made that fertility service providers discriminate against older women.²⁴ The principal argument against treating older women has been an allegedly unsatisfactorily low pregnancy rate and exceedingly high miscarriage rate, leading to allegedly unacceptably low ‘take-home baby’ rates.²⁵ However, such an analysis is not confirmed by our own center’s results with women over 40 years of age.²⁴

Table 2 summarizes the trend in previously reported age-specific pregnancy rates at our center, reflective of dehydroepiandrosterone (DHEA) supplementation for at least two months prior to IVF and aggressive ovarian stimulation with microdose agonist and high dosage gonadotropins. Clearly, the numbers demonstrate surprisingly high pregnancy rates, even more remarkable considering the high percentage of patients, previously refused further treatments elsewhere and advised to pursue egg donation.²⁴ Furthermore, DHEA has been shown to have a ‘rejuvenating’ effect on ovarian function.²⁶⁻²⁹ More specifically, it appears to increase oocyte/embryo yields²⁶⁻²⁹ and improve egg/embryo quality,²⁸ spontaneous pregnancy rates, pregnancy rates with IVF,²⁹ cumulative pregnancy rates,²⁹ and time to pregnancy.²⁹

How DHEA achieves all of these effects remains to be determined, though the observation that it takes four to five months to reach peak effects—a time period approximately equal to the completed follicular recruitment cycle—suggests an effect on follicular recruitment. Based on preliminary data, we suggested that DHEA may indeed reduce oocyte/embryo aneuploidy.³⁰

Such an interpretation and a more aggressive and proactive treatment approach toward older women is also supported by recently generated follow-up data on pregnancies, established under DHEA supplementation. Even though women with severely diminished ovarian reserves have been reported to demonstrate significantly higher miscarriage rates than age-matched women with normal ovarian reserve,³¹ in collaboration with Canadian colleagues, we were able to demonstrate that, at least in DHEA supplemented pregnancies, miscarriage rates were actually lower than in age-matched infertility controls and comparable to the average miscarriage rate (of approximately 15%) in the general population.³² As a large majority of miscarriages in older women are the consequence of aneuploidy, such a dramatic reduction in miscarriage rates would appear unachievable without a reduction in aneuploidy rates. Therefore, one can conclude that at least some DHEA effects may be the consequence of decreased aneuploidy.

National US IVF outcome reports for 2004 and 2005 for the first time recorded pregnancies in women at and above 46 years of age.^{33,34} Our own center’s oldest DHEA pregnancy so far occurred at 46 years and 11 months of age at time of conception, but unfortunately was miscarried after positive fetal heart

Table 2: Change in Clinical *In Vitro* Fertilization Pregnancy Rates of the Center of the Authors*

Patient Age (Years)	2004 (%)	2005 (%)	2006 (%) [§]
40	15.4	12.5	27.3
41	23.1	7.1	25
42	14.3	0	16.7
43	14.3	9.1	40
44	0	9.5	16.6
>44	0	–	20

*Table applies to women over 40 years of age. § First systemic dehydroepiandrosterone (DHEA) supplementation for women above age 40 years. The table demonstrates a significant increase in clinical pregnancy rates with introduction of more wide-scale DHEA supplementation in 2006. * Modified from Gleicher et al.²⁴ with permission.*

had been established and the patient had been transferred into obstetrical care. These data demonstrate slow but steady progress toward older ages at which infertility treatment with autologous oocytes can still succeed. Only a few years ago, 40 years of age was considered an almost prohibitive barrier to pregnancy success; 42 years of age was subsequently considered the upper limit of the achievable. Until recently, even our own center, which serves a much higher percentage of older women than most other IVF providers, considered a pregnancy at 45 years of age an exception. Although even today pregnancies at 45 years of age do not represent daily events, they have become frequent enough to be considered routine and it is the pregnancies at 46 years of age and above that create excitement. It seems obvious that, as our understanding of ovarian physiology and our technical abilities improve, progress will continue toward older and older female ages. Withholding care from older women creates a self-fulfilling prophecy because unless we try, we will never succeed.

Where We Have Gone Wrong

We previously noted that, remarkably, IVF pregnancy rates have historically steadily improved,³⁵ and that this positive trend should continue, unless untested systemic interventions into the IVF process are allowed. Unfortunately, untested systemic interventions, have been on the increase in recent years and, therefore, for the first time in the history of IVF threaten continuous improvements in pregnancy rates.

Untested systemic interventions can take various formats, e.g. legislation, such as the recently passed law in Italy that adversely influenced IVF outcomes.^{8,9} Paradoxically, government interventions into the practice of IVF have also been proposed by IVF practitioners³⁶ and, indeed, actively supported in a number of countries. Physician-driven systemic interventions can potentially be the most dangerous. They are usually propagated by well-meaning experts, convinced of sound hypothetical concepts that, considered overtly obvious, do not require serious experimental pre-testing before introduction into routine IVF practice. Two recent examples illustrate this point.

The Pre-implantation Genetic Screening Fiasco

PGS probably represents the best example of where the profession has gone wrong. Widely propagated as a tool to further improve pregnancy rates with IVF, the procedure superficially appeared to make considerable sense: with large percentages of human embryos being aneuploid, and thus not implanting or resulting in spontaneous pregnancy loss, what would appear to make more logical sense than the chromosomal screening of embryos prior to intra-uterine transfer? Logically, one would expect improved implantation rates and decreasing miscarriage rates from such an approach, resulting in

higher pregnancy rates and better take-home-baby rates.³⁷ The higher the risk for aneuploidy (i.e. the older the patient), the more effective PGS should be.

Therefore, who would be surprised that PGS swept the world of IVF and entered routine practice, even though a limited number of controlled studies failed to demonstrate demonstrable benefits?³⁸ The overwhelming assumed wisdom of the underlying concept resulted in all caution being thrown to the wind. Rather than demanding more and better studies, the profession reached the almost unanimous conclusion that those early data had to be wrong. However, data rarely lie. Re-analyzing early published data, for over two years we unsuccessfully attempted placement of a manuscript that criticized basic premises of PGS and its worldwide utilization. We argued that the proposed benefits of PGS would statistically hold up only if PGS (i.e. embryo biopsy) did not negatively affect embryo implantation chances (not a likely premise), PGS achieved a high degree of diagnostic accuracy in predicting embryo aneuploidy (with currently available techniques really unachievable), and if PGS was used to select embryos (i.e. requiring a large enough embryo pool to select from, thereby disqualifying most older women). Furthermore, we demonstrated in the manuscript that, barring full satisfaction of these three pre-conditions, PGS could actually adversely affect pregnancy chances. Our manuscript was finally accepted and published³⁹ after a Dutch study demonstrated that in older women PGS seems to reduce pregnancy chances with IVF.³⁹ Since then, many authoritative voices have spoken out against the uncontrolled utilization of PGS, declaring the procedure ineffective in improving IVF pregnancy rates and reducing miscarriage rates with IVF.⁴⁰ However, what has not yet been properly addressed is how the worldwide premature introduction of PGS into routine IVF practice could have occurred in the first place, and what lessons could be drawn from this unfortunate experience.

Our earlier noted calculations,³⁸ and the previously mentioned Dutch study,³⁹ leave little doubt that the utilization of PGS in inappropriately selected patients, at least in some instances, reduces pregnancy chances. These data also demonstrate convincingly in many patients that PGS, does not deliver on promised benefits of improvements in IVF pregnancy rates and decrease in miscarriages. Consequently, the premature introduction of PGS into clinical routine practice breached the primary tenet of healthcare: to do no harm. By reducing pregnancy chances without offering any visible benefits, PGS caused harm because it negatively affected the goal of infertility patients: to conceive.

This goal appears increasingly endangered by systemic interventions into standard IVF practices. It almost seems that parts of the medical profession no longer believe that the primary goal of infertility treatment—and consequently, the overwhelming obligation of physicians—is to protect a patient's desire for pregnancy. Instead, as described above, systemic interventions are introduced under hypothetical assumptions of benefit willingly accept proven reductions in pregnancy chances. In other words, once again the basic tenet of healthcare—to do no harm—is violated since a certain detriment (reduction in pregnancy chance) is accepted based on an only hypothetical benefit that, as PGS demonstrated, later may or may not be proved correct. The ethical conduct of medical practice, of course, prohibits such situations from occurring. Treatments without confirmed appropriate risk–benefit considerations should be considered experimental, and it is unethical to introduce them into standard treatment protocols.

Unfortunately, PGS is not the only recently introduced systemic intervention into standard IVF practice that reduces pregnancy rates without delivering

on promised benefits. Other examples abound and, because of their considerable potential for lost chances to conceive, for the first time in the history of IVF they endanger the steady and continuous improvement in IVF pregnancy rates worldwide.

Single-embryo Transfer

The most blatant example is the widely propagated concept of SET,⁴¹ which similar to PGS is based on false assumptions and incorrect statistical considerations and was initially proposed to improve IVF outcomes. The principal arguments in favor of SET were:

- twin pregnancies represent higher outcome risk than singletons;
- therefore, twin pregnancies should be avoided—even at the expense of reducing pregnancy chances; and
- SET avoids the risk for twins pregnancies.

While this line of thought on first impression (such as the rationale for PGS) appears logical, on closer examination it actually proves statistically fatally flawed. Nobody can argue with the fact that twin pregnancies carry higher risks than singletons. However, such comparative data reflect an obstetrical treatment paradigm of spontaneous conception and *post factum* risk analysis. Infertility treatment reflects a different treatment paradigm, where a new infertility patient prospectively views cumulative risks of achieving desired goals. For a large majority of infertility patients, the desired goal is more than one child; therefore, those who want two children, theoretically at least, have the option of choosing one twin or two singleton deliveries. Controlling for desired outcome (i.e. two children), the statistically correct risk comparison between twin and singleton deliveries in such patients is not between one singleton and one twin, but between two singletons and one twin delivery.^{42–44}

When this is completed, twin pregnancies suddenly no longer demonstrate clinically relevant excessive risks, thus depriving SET of alleged outcome benefits in comparison with two-embryo transfer (2ET) and, with it, of any rationale.⁴² Indeed, since SET reduces pregnancy chances in comparison with 2ET,⁴⁵ it once again exposes IVF patients to reduced pregnancy chances without compensatory benefits and, therefore, breaches the 'do no harm' covenant, paradoxically historically used by proponents of SET in support of the procedure. Therefore, we consider SET contraindicated, unless patients are desirous of only a single child and/or have obstetrical contraindications against twin deliveries.

In some European countries, the concept of SET is directly legislated (Belgium), or indirectly through government-appointed bodies (UK). If the medical establishment can be so obviously wrong in reaching recommendations, how can one expect government to be wiser? However, professional medical opinion can shift quickly. As the experience with PGS has demonstrated, publication of a single study can be decisive.³⁹ To reverse legislation, in contrast, takes much more effort and time, suggesting once more that medical practice patterns should not be subject to government interventions, but rather should remain under professional controls.

Other Bad Ideas

In Europe especially (also the birthplace of SET), the increasing popularity, and untested introduction into practice, of so-called low-intensity, natural or patient-friendly IVF cycles⁴⁶ raises the specter of further declines in

pregnancy rates.⁴⁷ All of these newly proposed IVF approaches have one feature in common: they reduce pregnancy rates in comparison with standard IVF cycles, and do so in return for only alleged benefits to patients. However, infertility patients have repeatedly gone on record clearly indicating that nothing is as important to them as achieving pregnancy.⁴⁸ Therefore, it seems inappropriate for professionals to assume that any of the above-noted IVF variations, in view of significantly reduced pregnancy chances, will find a receptive audience. Indeed, unless any of these systemic interventions into current IVF practices can be demonstrated to offer patients clear benefits over current standard IVF practices, they with great likelihood (and rightly so),⁴⁷ will be widely rejected by the public.

Patients Are Entitled to Maximal Pregnancy Rates

All of this raises the obvious question of whether infertility patients, in consideration of untested systemic interventions into standard IVF care, should still be considered to receive best possible medical care. Since in many of the above-outlined circumstances such interventions are reducing pregnancy chances without compensatory benefit, one is left wondering whether the many currently espoused directions of IVF practice really reflect the best interests of the patients.

We do not believe so.⁴⁸ In seeking out infertility care, patients are, first and foremost, entitled to achieving their principal goal: conception. Fully accepting the notion that a physician's first responsibility is to do no harm, we in principle agree that IVF should minimize risks to mothers and offspring. However, this does not absolve physicians from providing patients with their respective maximally achievable pregnancy chances, as long as doing so does not create unreasonable risks, which for the patient exceed the potential benefits of childbirth. In other words, as for any medical intervention, an appropriate and correct risk–benefit assessment is essential. However, alleged risks and benefits have to reflect appropriately conducted studies and cannot be assumed.

As PGS and SET concepts demonstrate well, even well thought-through hunches by prominent authorities are still only hunches, and can be misleading. Until such notions are replaced by factual data, all of the above discussed systemic interventions into standard IVF practices should therefore be considered experimental and should be offered only under experimental study conditions and with appropriately executed informed consents. Any other approach should be considered unethical and a significant risk to the continuous improvement of IVF outcomes. ■

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