CHAPTER

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The Ovarian Factor in Assisted Reproductive Technology

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Abbreviations

ACTH adrenocorticotropic hormone
AFC antral follicle count
AMH anti-Müllerian hormone
ART assisted reproductive technology
ASRM American Society for Reproductive Medicine
CDC Center for Disease Control
COH controlled ovarian hyperstimulation
DHEA dehydroepiandrosterone
DOR diminished ovarian reserve
eSET elective single embryo transfer
ESHRE European Society for Human Reproduction and Embryology
FOR functional ovarian reserve
FSH follicle-stimulating hormone
GC granulosa cell
GnRH gonadotropin-releasing hormone
GSC germ-line stem cell
hCG human chorionic gonadotropin
HGH human growth hormone
hMG human menopausal gonadotropin
HPAA hypothalamic-pituitary-adrenal axis
ICM inner cell mass
IVF in vitro fertilization
IU international units
LFOR low functional ovarian reserve
LH luteinizing hormone
mDNA mitochondrial DNA
nDNA nuclear DNA
OHSS ovarian hyperstimulation syndrome
OI ovarian insufficiency
oPOI occult primary ovarian insufficiency
OR ovarian reserve
PB polar body
PCOS polycystic ovary syndrome
PGS preimplantation genetic screening
PGT-A preimplantation genetic testing for aneuploidy
POA premature ovarian aging
POR poor ovarian response
POI primary ovarian insufficiency
PVS perivitilin space
SART Society for Assisted Reproductive Technology
SHBG sex hormone binding globulin
SOI secondary ovarian insufficiency
TSH thyroid-stimulating hormone
TOS total oocyte score
ZP zona pellucida

DEFINITIONS

Definition of Ovarian Factor

Embryos are typically created by successful fertilization of one oocyte by one spermatozoa. The resulting union of maternal and paternal nuclear haploid genomes (nDNA) establishes the newly diploid nDNA of the offspring embryo. It is always accompanied by an exclusively maternal mitochondrial genome (mDNA), as the paternal mitochondrial genome (spermatozoa) is lost soon after entering the maternal cytoplasmic oocyte microenvironment. Egg and sperm at fertilization, therefore, are not "equal" partners. As host-cell for the union of maternal and paternal genomes, the oocyte dominates the biology.

Mitochondrial genetic diseases, consequently, are only inherited from mothers, and embryo quality is largely determined by oocytes rather than sperm. Among all contributing factors to reproductive success, the "ovarian factor" is, therefore, the most important and, likely, most multifaceted. It is defined by nDNA, mDNA, and other cytoplasmic constituents of importance; but also by folliculogenesis, the months-long maturation process of follicles and oocytes in the ovarian microenvironment, still by investigators not given appropriate attention.

The "ovarian factor" is also highly age-dependent, still believed to be characterized by finite numbers of oocytes at their most primitive stage (in primordial follicles) in ovaries since embryonic life [some investigators have, although, raised the possibility of neoformation of follicles and oocytes from germ-line stem cells (GSCs)], and steadily declining in quantity and quality. As women age, oocytes are, therefore, presumed to "age" in parallel,

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reflected in decreasing functionality of nuclear and cytoplasmic components and in progressively declining egg quality. The presumed consequences are increases in meiotic chromosomal abnormalities, which increase with advancing female age from as low as 10%–20% to in excess of 60% [1].

We suggested a number of years ago a somewhat more complex concept of ovarian aging (the "CHR concept of ovarian aging"): primordial follicles at resting stage are primitive structures with almost no metabolic activity. Because they lack contact with their environment, they until recruitment, therefore, are in the external capsule of ovaries largely isolated from environmental influences. Only once recruited into folliculogenesis, do follicles/oocytes establish increasing metabolic interdependence with their microenvironment and, therefore, become more vulnerable to environmental influences.

This concept of ovarian aging, therefore, suggests that primordial follicles experience only minor damage while, often for decades, existing at resting stage in aging ovaries. Once recruited, however, they become subject to the toxic effect of an aging ovarian microenvironment, in which they undergo maturation over weeks to months. The damaging culprit in declining oocyte quality with advancing female age in this model is, therefore, not the time oocytes spent in the ovarian capsule as primordial follicles but the aging ovarian environment, in which they now must spend weeks to months of maturation, while migrating from the ovarian capsule inward toward the medulla [2].

Differences between above-described two hypotheses of oocyte aging are of considerable clinical importance: under the traditional hypothesis of ovarian aging, it is difficult to imagine that oocytes damaged by time may, still, be pharmacologically rescuable and repairable. Clinical interventions to improve oocyte quality in older women would, therefore, appear moot. Since annual US National Assisted Reproductive Technology (ART) outcome date demonstrate that reproductive endocrinologists rarely choose to treat women above age 42 with use of their own eggs, it is reasonable to assume that most of them still subscribe to this opinion.

An aging ovarian microenvironment, however, could potentially still be subject to successful therapeutic interventions. The "CHR concept of ovarian aging" arose after observing beneficial effects of androgen supplementation on ovarian function, resulting in larger oocyte yield and improved oocyte quality [3,4]. This concept, therefore, recognizes the possibility that therapeutic interventions into early stages of folliculogenesis, which reconstitute physiological conditions of "younger" ovarian microenvironments and, therefore, improve the conditions of follicular maturation, may improve oocyte numbers and quality in older women. The "CHR concept of ovarian aging" creates the opportunity for pharmacological interventions into early stages of follicle maturation to benefit older women who, still, wish to conceive with use of their own oocytes.

Since males produce fresh sperm into advanced ages, paternal aging is of less concern, although recently reported data suggest that the decline in sperm quality with advancing male age may be more severe than has been so far appreciated.

Definition of ART

ART encompassed a variety of treatments, techniques, and technologies. In this chapter, we will concentrate on the process of in vitro fertilization (IVF). IVF, normally, encompasses controlled ovarian hyperstimulation (COH) with fertility drugs, oocyte retrieval, IVF of oocytes with sperm in the laboratory, culture of resulting embryos, and embryo transfer into the uterus, either on day 3 after fertilization (cleavage stage) or on days 5–7 (blastocyst stage).

COH in a large majority of cycles utilizes at different dosages gonadotropin stimulation, whether in form of injectable follicle-stimulating hormone (FSH) and/or human menopausal gonadotropin (hMG), which is a mixture of FSH and luteinizing hormone (LH). COH can also involve oral medications like clomiphene citrate and/or aromatase inhibitors, like letrozole, or a combination of orals and injectables. A small minority of IVF cycles utilize the natural cycle, thereby avoiding COH.

Medication dosages are determined by a patient's ovarian reserve (OR), which is generally understood as the estimated number of remaining follicles/oocytes in ovaries [2]. The lower the OR, the higher the required medication dosages that will be administered to obtain adequate ovarian responses to stimulation. Since OR in women declines with advancing age, medication requirements for COH usually increase as women age.

A woman's OR is made up of two distinct pools: a large majority of follicles/oocytes are in the so-called resting pool of primordial follicles, while at any given moment just a small proportion is in the so-called growing follicle pool (follicles after recruitment). Only the latter can be assessed with reasonable accuracy. In representation, and in contrast to the total OR that includes the resting follicle pool, we, therefore, describe this growing follicle pool in this chapter as the so-called functional ovarian reserve (FOR). Women with abnormally low numbers of growing follicles have low functional ovarian reserve (LFOR). The size of the resting follicle pool can be roughly estimated because it is usually proportional to the growing follicle pool [2].

Low OR (LOR) also called diminished OR (DOR) and LFOR are widely used terms, although, unfortunately, not well defined. It is important to recognize how important age is in determining what represents normal OR:

what may be a perfectly normal OR at age 43, would be a LOR/LFOR at age 25. OR determinations should, therefore, be based on age-specific values of OR assessment tools, such as FSH, anti-Müllerian hormone (AMH), and antral follicle counts (AFCs) [2].

Another widely used term in the literature, mostly defined by the so-called Bologna criteria [5], is poor ovarian response (POR). Patients who produce smaller than expected oocyte yields are considered poor responders. Women with LOR/LFOR usually demonstrate POR. Since women become more resistant to ovarian stimulation with advancing age, like LOR/LFOR, POR also varies with female age. Although both represent distinctly different descriptions of ovarian function, LOR/LFOR and POR are often, nevertheless, used almost interchangeably. While diagnoses of LOR/LFOR and POR in most patients indeed overlap, they are clinically distinctively different: a diagnosis of LOR/LFOR (and, therefore, assumption of POR) can be reached in advance of even first IVF cycles; a diagnosis of POR, however, is only possible after at least one prior COH.

This is, however, not the only reason why these two terms should be considered distinct. POR is an even more subjective diagnosis than LOR/LFOR since unexpectedly low responses to ovarian stimulation may also be caused by nonovarian causes. Specifically, iatrogenic interferences, like medication errors, wrong medication dosing and patient obesity may lead to a mistaken diagnosis of POR. LOR/LFOR, in contrast can always be assessed objectively by measuring FSH, AMH, and/or AFCs. Although the diagnosis of POR is widely used in the medical literature, we do not favor this diagnosis for either clinical or for study purposes, preferring the diagnosis of LOR/LFOR.

Accurate and objective age-specific assessments of OR are essential in correctly defining the "ovarian factor" in infertile couples. Those then permit determinations whether age-specific ORs are normal, low, or high. Such determinations, in turn, often lead to diagnoses of premature ovarian aging (POA), also called occult primary ovarian insufficiency (oPOI) if OR is abnormally low or to the diagnosis of polycystic ovary syndrome (PCOS) if OR is unusually high [2]. Accurate OR assessments in advance are, of course, also of crucial importance in correctly individualizing and maximizing ovarian stimulation protocols for patients.

The number of different ovarian stimulation protocols for IVF has proliferated in recent years, with some being of questionable efficacy [6]. A detailed review of all of them would exceed the framework of this chapter. Relevant details will, however, be discussed in —sections "What Controls the Ovary?," "How the Ovary Controls Treatment Success in IVF," and "Affecting Ovarian Performance."

Beyond new COH protocols, routine IVF over the last decade has undergone considerable changes. A variety of so-called add-ons to standard IVF have been integrated, many increasingly questioned in their clinical utility [7]. Their discussion would also exceed the framework of this book chapter. Since so much depends on patient selection, outcome assessments in IVF are highly complex [8]. They, therefore, are often subject to subconscious or conscious manipulations [9]. When assessing IVF cycle outcomes, and one cannot review the "ovarian factor" in ART without paying close attention to IVF treatment outcomes, considerable care is, therefore, required to represent data correctly. Unfortunately, that has not always been the case in recent years, often misleading not only the public but also fertility practitioners. This chapter will, therefore, make special efforts to be transparent, and reflect IVF cycle outcomes correctly and objectively.

OVARIAN RESERVE

Physiology of Ovarian Aging

Fig. 1 demonstrates how a woman's OR evolves from intrauterine life through menopause: peak follicle numbers in ovaries are reached at approximately 24 weeks intrauterine life, when ovaries contain approximately 7.0 million follicles. They then, however, rapidly decline in the steepest period of follicle loss to 3.5 million at time of birth, only to further diminish to <1.0 million by puberty. Paradoxically, the slowest period of decline occurs during reproductive years, and by menopause only a few hundred are left.

Approximately 90% of women of all racial/ethnic backgrounds follow the ovarian aging curve outlined in Fig. 1 ("ovarian age" being defined by remaining follicles/eggs in ovaries). They are considered to have normal OR/FOR. If excessively high-AMH values are seen, OR/FOR is considered excessively high, suggestive of a PCOS diagnosis, even though current international diagnostic criteria, still, do not consider abnormally high age-specific AMH values as diagnostic of PCOS [10].

Approximately 10% of women demonstrate evidence of abnormally low age-specific follicle/egg numbers, and are considered to suffer from LOR/LFOR. Within this group of women, c. 1% reach a diagnosis of primary ovarian failure (POF), now also called primary ovarian insufficiency (POI); while the remaining 9% only reach the stage of POA, also called oPOI [2].

Diagnosing Normal vs Abnormal OR

Assessing whether an infertile woman has normal, high, or LOR/LFOR, should, therefore, be an initial step in any infertility evaluation. What represents normal OR is, however, not defined by single data points but by ranges. Unfortunately, the concept of age-specific OR

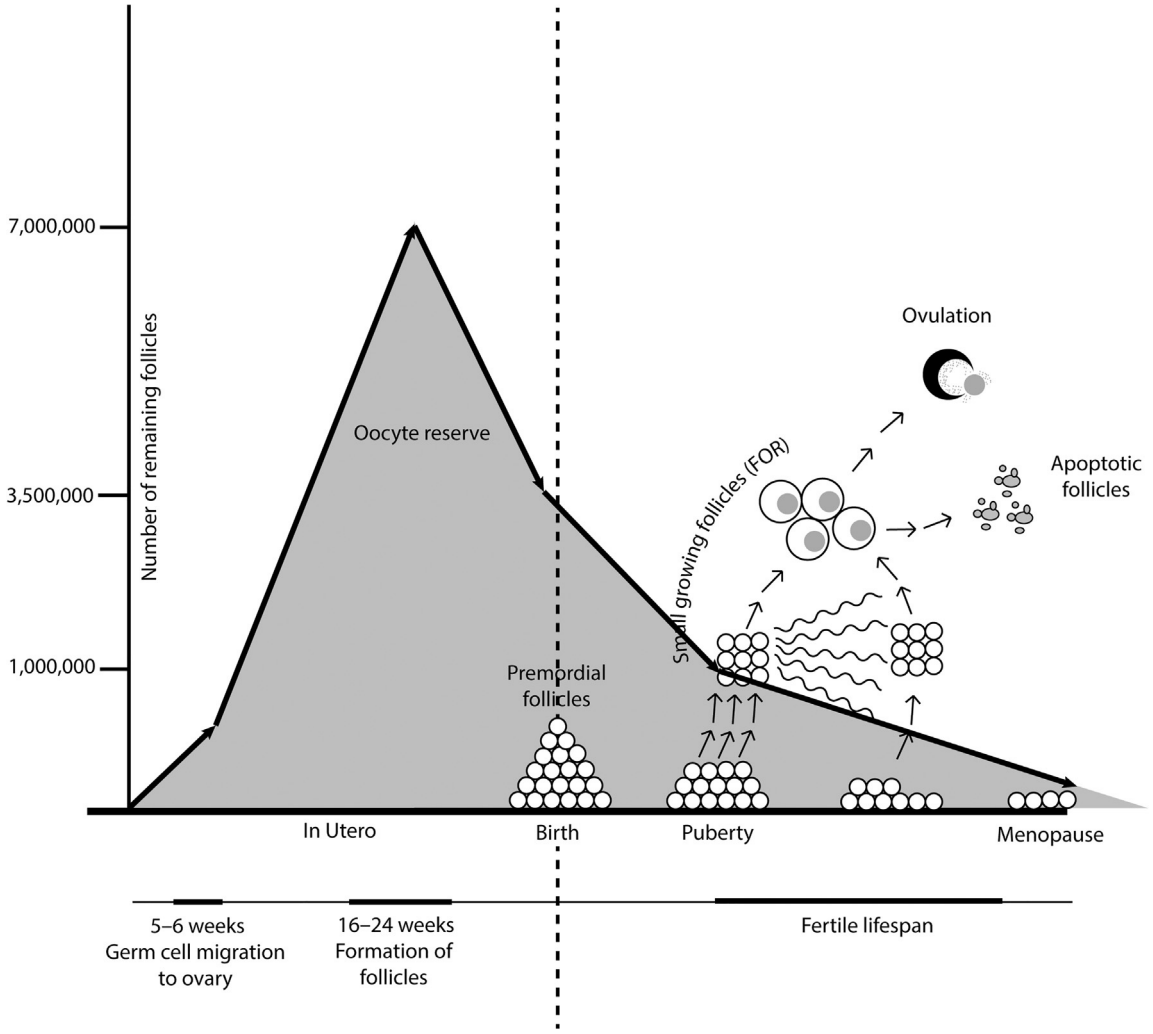


FIG. 1 Ontogeny of ovarian reserve (OR). The figure demonstrates that ovaries contain peak follicle numbers at approximately 24 weeks intrauterine life, reach c. 3.5 million at birth, only <1.0 million at puberty and by menopause only a few hundred.

assessments via such classical diagnostic parameters as FSH, AMH, and AFCs, is only slowly permeating fertility care [11,12]. This may surprise the reader since it seems such a simple, yet logical concept: with advancing age, steadily declining AMH values and AFCs and rising FSH levels must have different meanings at different ages (Fig. 2).

When, as many commercial laboratories still do, early follicular phase FSH ranges up to 10.0–12.0 mIU/mL are reported as "normal," those include women of very advanced ages. Consequently, an FSH of 10.0 mIU/mL at age 44 may, indeed, still reflects normal OR; but a value of 10.0 mIUI/mL in a 34-year-old women must be viewed as abnormally high and, therefore, suggestive of LOR/LFOR. Age specificity also applies to AMH and AFCs, although both of these OR parameters, of course, decline with advancing age. Simple baseline assessments of FSH, AMH, or AFCs, if age specific, therefore, permit with considerable accuracy prospective determinations of OR and clinical differentiation between women with normal OR, likely PCOS or LOR/LFOR.

Fig. 2 demonstrates age-specific FSH and AMH levels, established at our center several years ago, based on 95% confidence intervals of the center's infertile patient population. They have been useful but must be used with a degree of caution because, if obtained in normally fertile women, FSH would, likely, be marginally lower and AMH marginally higher. We are unaware of age-specific FSH or AFC curves obtained in fertile patients but know of one publication of age-specific AMH values in general populations [13].

Why OR Determines Ovarian Stimulation

Because natural cycles are usually unifollicular and, therefore, not very productive in oocyte yields, COH represents an essential part of most IVF cycles. After female

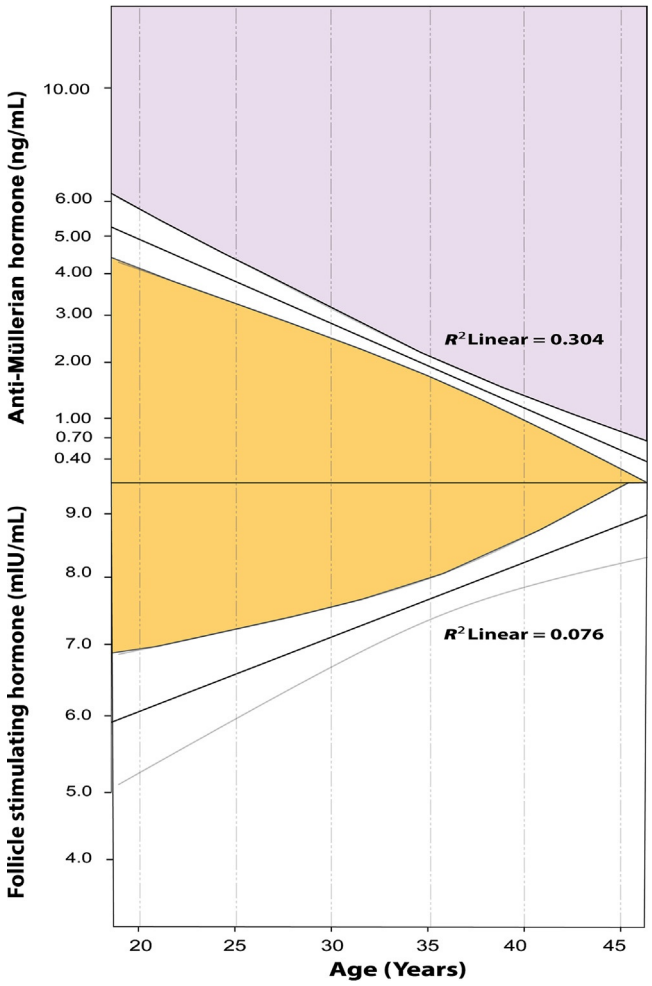


FIG. 2 Age-specific anti-Müllerian hormone (AMH) and follicle stimulating hormone (FSH) levels, as utilized at our center. The figures demonstrate age-specific 95% CIs for FSH (based on an infertile patient population) and AMH, allowing for prospective determination of OR. Here demonstrated ranges, likely slightly exceed FSH and underreport mildly AMH levels in comparison with ranges in a fertile population. *Copyright, The Center for Human Reproduction, with permission.*

age, oocyte and embryo numbers are the most important predictors of pregnancy and live birth rates [14], although others have suggested that there is an "ideal" range of oocyte numbers, below and above which IVF outcomes are diminished. Excessive oocyte numbers are also associated with an increased risk of ovarian hyperstimulation, and the ovarian hyperstimulation syndrome (OHSS) is one of the most feared clinical complications of IVF [15].

A principal goal of planning any IVF cycle is, therefore, choice of best ovarian stimulation protocol. This, of course, if possible, should be done prospectively to avoid understimulation or overstimulation even in first IVF cycles. Various prediction algorithms have been published based on patient age, FSH, and AMH levels but have not achieved significant following.

At our center, hyperstimulation is almost never a risk since we serve a very adversely selected patient population, mostly involving women with LOR/LFOR. In such patients, the center's standard protocol is a microdose agonist protocol, originally proposed by Surrey et al. for "poor responders" [16], with maximal gonadotropin dosages [in a combination of FSH and LH]. In patients with very low OR, we recently moved to early retrieval at smaller lead follicle sizes, when prevention of spontaneous ovulation is no longer necessary and microdose agonist can be omitted [17].

Our center's standard protocol for young patients with normal age-specific OR and for highly selected egg donors is still a long-agonist protocol with an appropriate gonadotropin stimulation (usually a combination of FSH and LH in a single hMG product). Patients and egg donors, based on very high-AMH values suspected of PCOS, are also stimulated in long-agonist protocols, though with lower gonadotropin dosages.

As noted earlier, under current international definitions, including Rotterdam criteria, AMH values are still not included in diagnostic criteria that define PCOS. Clinical practice has, however, embraced abnormally high-AMH levels as a strong indication of likely PCOS and, in association with IVF, as a risk marker for large oocyte yields and potential OHSS risk [10]. Combined, age-specific FSH and AMH levels, even without complex predictive algorithms, therefore, reliably differentiate between women with low, normal, or high OR, and allow appropriate choice of stimulation protocols.

This is a crucial reason why we, as previously noted, prefer the diagnostic definition of patients by objective OR parameters to the still widely used definition of patients as "poor responders," which is retroactive and, therefore, dependent on at least one prior ovarian stimulation cycle. Most women with LOR/LFOR will, obviously, also be poor responders, and through testing of their FOR with FSH and AMH, can already in first IVF cycles be well defined in their expected responses to stimulation.

DEFINITION OF TREATMENT SUCCESS IN ART

The purpose of ART is pregnancy and, ultimately, birth of healthy term offspring. What outcomes should be viewed as treatment successes has, however, remained controversial. Initially proposed in Europe, but recently also increasingly embraced in the United States, the opinion that only singleton healthy births should be considered treatment successes in IVF has been gaining momentum. Under this concept, all multiple pregnancies (including twins), therefore, are considered adverse IVF outcomes and should be avoided [18]. We have strongly

opposed this view, and readers interested in the ongoing debate on this subject are for further detail referred to a recent publication [19].

As previously pointed out, since OR declines with advancing age and egg numbers and egg quality deteriorate (leading to declines in embryo quantity and quality), treatment success in IVF is age dependent. Pregnancy chances per transferred embryo (i.e., implantation rates), therefore, also decline. In addition, live birth chances are further reduced and miscarriages increase because of increasing meiotic oocyte aneuploidy. To compensate for declining implantation rates, it is generally recommended to increase the number of embryos that are replaced into the uterus [20].

Under federal law, all US IVF centers are mandated to report IVF cycle outcomes to the Center for Disease Control (CDC). Since nonreporting IVF centers are not penalized, the relatively small number of nonreporting centers has recently increased. A large majority of centers, however, submit annual reports, which are posted after some vetting by the CDC for public consumption, and are subject to potential audits. The Society for Assisted Reproductive Technology (SART), a daughter society of the American Society for Reproductive Medicine (ASRM), manages a parallel IVF outcome reporting data set. Since SART in recent years significantly improved its reporting system, its annual reports now increasingly divert from published CDC reports.

Both systems, however, still misinform the public about individual clinic performances. The principal motivation for Congress to establish the reporting mandate was to provide accurate clinic outcome information about IVF to the public. Its purpose, therefore, has not been fulfilled. Reasons are incorrect data analyses by CDC staff [9] and loopholes in cycle reporting rules for individual centers, slowly addressed by SART but not yet by the CDC. Going forward, CDC and SART data, therefore, will, likely, diverge even more, thereby further confusing the public.

Currently, existing loopholes allow selective cycle reporting, offering IVF centers the option of intentionally excluding poorer prognosis patients [21]. Centers that do this, gain significant reporting advantage by artificially inflating their outcomes. A small number of outlier centers follow such practices to the extreme. Unknowingly, the public, unfortunately, amply rewarded these centers for their misrepresentations since they, proportionally, doubled their market share of IVF cycles (12%) in comparison with the large majority of IVF centers, which did not abuse the system to similar degrees (6%) [9].

Reliable outcome reporting of IVF cycles is not only important for the public. It also is a cornerstone for progress in the field. Since establishment of IVF as a routine clinical procedure in the early 1980s, IVF outcomes have steadily improved (see US live birth rates over the decades in Fig. 3). Remarkably, those improvements, however, ceased in 2004, plateaued, and over the last few years actually declined. As a result, 2014 national live birth rates in the United States were below 2004 rates. Most regions around the world reported similar, and sometimes even worse declines in live IVF births over the last decade [22]. In recent years observed actual declines, at least in the United States, are, likely, even more substantial than demonstrated in Fig. 3 since reported live birth rates reported to the CDC, as noted above, often are exaggerated.

Reliable outcome reporting is, however, essential to assessments of treatment success. Over the last decade, IVF practices changed significantly all over the world, and not always to the benefit of IVF outcomes. Inadequately vetted add-ons to IVF, recently received increasing attention in the medical literature [7] and in lay media [23]. They, likely, are at least partially responsible for declining IVF live birth rates over the last decade [24].

In the industrialized world, the "graying" of patient populations undergoing IVF may also contribute to this problem [25]. As the average age of patients is increasing, older women with poorer outcome prognoses accumulate in IVF centers, and often require multiple IVF cycles. Younger and, therefore, better-prognosis patients, in contrast, quickly conceive and rapidly exit IVF programs. Our center, which among reporting US centers serves

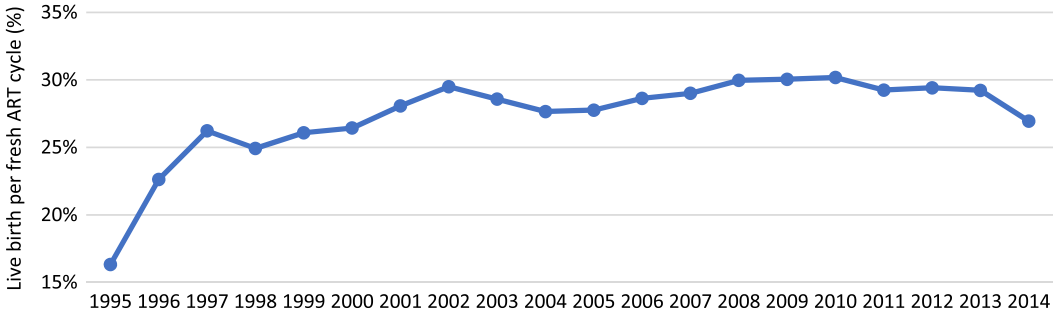


FIG. 3 US live birth rate after fresh in vitro fertilization (IVF) cycles. The figure demonstrates, more or less, steady improvement in US live birth rates over the years but a significant drop over the last few years, which brings the 2014 rate to below the 2004 rate. *After Gleicher N, Kushnir VA, Barad DH. Commoditization and industrialization of IVF is responsible for worldwide declining IVF birth rates. 2018 [submitted for publication] with permission.*

the by far oldest patient population, in 2017 for the first time exceeded a median age of 43 years for IVF cycle starts. Although only 5 years ago women above age 45 were only rarely treated with use of their own eggs, they in 2017 represented approximately 30% of our patient population.

With rising patient ages in IVF, relevance and relative importance of the "ovarian factor" is progressively rising in parallel. One, indeed, now can argue that ovarian aging, and with it the "ovarian factor," currently represent the single most important cutting-edge ART issue, requiring national attention.

WHAT CONTROLS THE OVARY?

The Hypothalamic Pituitary Axis

As in detail discussed elsewhere in this volume, a main function of the hypothalamus, a region in the lower part of the brain surrounding the third ventricle, is to maintain homeostasis of the body. It does so to a significant degree by controlling the autonomous and motor nervous systems, as well as behavioral and endocrine systems. Recent research has clarified many previously unknown functions of hypothalamic neurohormones which, secreted by parvocellular neurosecretory cells, control anterior pituitary tropic hormones which, in turn, control peripheral endocrine organs, including thyroid gland [thyroid-stimulating hormone (TSH)], breast (prolactin), the adrenal cortex [adrenocorticotropic hormone (ACTH)], liver and cells throughout the body [human growth hormone (HGH)], and female and male gonads (FSH and LH).

In discussing the "ovarian factor" in ART, the hypothalamic pituitary axis of gonadotropin-releasing hormone (GnRH) in the hypothalamus, FSH and LH in the anterior pituitary, and estrogen (as well as progesterone) in the ovary, is widely considered the essential hormonal building block of female fertility, controlling ovarian function. This axis, in recent years, has also been demonstrated to regulate onset of puberty through newly discovered kisspeptinergic neurons in the hypothalamus that release kisspeptin, a peptide that regulates the reproductive cycle by maintaining a feed-back loop with ovaries [10]. Kisspeptin is a very powerful stimulant of the GnRH-FSH/LH-estradiol axis.

GnRH, a decapeptide, is produced by neurons in the infundibular nucleus and the so-called preoptic area of the hypothalamus. It is then transported to the gonadotrophs of the anterior pituitary, which represent <15% of that region's cell population, and are stimulated to produce FSH and LH, the two principal gonadotropins controlling ovarian function. As its name suggests, FSH in principle has the function of stimulating follicle growth. FSH acts on small follicles synergistically with testosterone during early stages of maturation but has much greater effect during the last 2 weeks of folliculogenesis, when follicles reach maximal FSH sensitivity and FSH drives the follicular phase of the menstrual cycle. FSH is then inhibited by the corpus luteum in the luteal phase through production of inhibin. The principle function of LH is induction of steroidogenesis and the triggering of ovulation, although recent evidence suggests that, especially in older women, LH may also benefit COH [26,27].

The strong control of ovarian function by gonadotropins was first explored therapeutically in the late 1950s and early 1960s in groundbreaking studies by Carl Alex Gemzell in Sweden [28] and Bruno Lunenfeld in Israel [29], both considered the fathers of gonadotropin supplementation in infertile women, a treatment that initiated modern infertility care, and became a cornerstone of COH in IVF. COH with gonadotropins, till today has remained mostly unchanged. Pharma companies, however, modernized production by moving from the extraction of gonadotropins from the urine of postmenopausal women to recombinant technologies, and by aggressively promoting gonadotropin products which either exclusively or almost exclusively contain FSH.

In contrast, the original gonadotropin product that dominated the infertility world during the early years of IVF, was a hMG product (Pergonal), in equal parts FSH and LH. Its removal from the market is still decried by "old-timers." Contrary to claims by the pharma industry, we, still, interpret the published literature as suggesting that, in average populations, hMG products offer better pregnancy and live birth rates in comparison with stimulation with pure FSH. This issue, however, has remained controversial.

A second family of drugs in wide use in IVF is GnRH agonists and antagonists. Both were initially introduced to prevent spontaneous premature ovulation in gonadotropin-stimulated IVF cycles. Reaching the market after agonists, antagonists were promoted by industry as more "patient friendly" and noninferior regarding IVF cycle outcomes since they shorten COH cycles and reduce required overall gonadotropin dosages. We, however, observed in our patient population small, but statistically significant declines in pregnancy and live birth rates with antagonist in comparison with agonist cycles (Gleicher et al., unpublished observation). Especially in women with LOR/LFOR, we, therefore, avoid antagonists.

To go into more detail regarding expanding indications for these drugs over the last decade, would exceed the framework of this chapter. Only so much: since antagonists permit better cycle planning, including increasingly popular cycle-free weekends, popularity of GnRH antagonists stems not only from being more "patient-friendly" but also from being more "physician-friendly."

Agonists are now also used [in place of human chorionic gonadotropin (hCG)] to trigger ovulation. In this context, they also have become very useful in reducing OHSS risks in women like PCOS patients, who are excessive responders to stimulation [30].

The Newly Discovered Adrenal-Ovarian Axis

The hypothalamic-pituitary axis is often also called the hypothalamic-pituitary-adrenal axis (HPAA) because the adrenals were the first peripheral endocrine organ to be investigated within the hypothalamic control system of peripheral endocrine functions. Adrenals produce approximately half of a woman's androgens (the other half comes from ovarian theca). The discovery that normal ovarian follicle maturation at small growing follicle stages requires adequate intraovarian testosterone concentrations to mediate androgen receptor activity on granulosa cells (GC) [31,32], connected androgen production by the zona reticularis of adrenals, suddenly, to ovarian function because hypoandrogenism, at practically all ages, is a feature of LOR/LFOR [33,34]. Like hyperandrogenism in PCOS, hypoandrogenism in LOR/LFOR patients can be in origin ovarian, adrenal, or combined. Infertile women with clearly adrenal hypoandrogenism have recently been described in a hypoandrogenic PCOS-like phenotype [35].

With insufficient androgen production in the zona reticularis of adrenals, peripheral androgen levels decline. Testosterone and dehydroepiandrosterone sulfate (DHEAS), the only androgen hormone almost exclusively produced by adrenals, will, therefore, both be abnormally low. As in PCOS patients abnormally high testosterone and DHEAS levels suggest adrenal overproduction of androgens, so does low peripheral testosterone in association with low DHEAS in women with LOR/LFOR and in the above-noted hypoandrogenic PCOS-like phenotype suggest deficient adrenal androgen production.

Multiple animal models have demonstrated that, without adequate androgen levels, primary and small antral stage follicles, will mature poorly and/or completely arrest. Consequently, fewer follicles/eggs reach maturity, and those that do, are of poor quality [31,32,36].

Recognition of importance of androgens for small follicle growth led to therapeutic supplementation of hypoandrogenic infertile women with either oral dehydroepiandrosterone (DHEA) or transdermal testosterone [37]. In supplementing infertility patients with androgens, it is important to remember that androgens primarily only benefit small growing follicles. Those, however, still require weeks to months to reach gonadotropin sensitivity. Androgen supplementation, therefore, must be initiated at least 6–8 weeks before IVF cycle start and further increases in statistical effectiveness up to 3–4 months before reaching a plateau.

Whether hypoandrogenism in infertile women is of adrenal or ovarian origin is prognostically of great importance: when caused by ovarian theca insufficiency, it usually denotes truly "burned out" ovaries, and androgen supplementation, at best, will only marginally improve ovarian function. In cases of adrenal androgen insufficiency, ovarian function may, however, still, be intact. In such cases, low androgens in the ovarian microenvironment can lead to interruption of normal folliculogenesis, and adequate presupplementation of androgens to achieve appropriate testosterone levels (between 30 and 60 ng/dL or 1–2 nmol/L) prior to IVF cycle initiation may revive ovarian responses in terms of oocyte yields and oocyte quality in such patients [37]. Whether DHEAS is low, normal, or high in hypoandrogenic infertile women undergoing IVF is, therefore, of great prognostic significance.

What constitutes normal testosterone values greatly varies since androgen assay systems utilized by laboratories differ significantly. Interpretation of results is further complicated by nonage-specific normal laboratory ranges, even though normal testosterone and DHEAS levels greatly vary depending on age. All androgens decline rapidly after age 40. Sex hormone binding globulin (SHBG) levels help in evaluating whether a patient's testosterone is in range since these two laboratory tests usually move into opposite directions: a patient with low testosterone will demonstrate high SHBG and vice versa (some caution is indicated since thyroid and other medical problems may affect SHBG). Hypoandrogenic patients with abnormally high SHBG will, therefore, usually normalize SHBG levels as testosterone normalizes. We, therefore, use SHBG as a secondary parameter in assessing when patients have reached balanced androgen levels.

Adrenal hypoandrogenism can produce a surprisingly similar clinical presentation to POA/oPOI, and, with significantly elevated FSH and undetectable AMH, even mimic POF/POI. As already noted before, this is explained by severe hypoandrogenism arresting follicle growth which, in turn, reduces GC mass and, therefore, estradiol production. Negative feedback then raises FSH. Although relative rare, we have seen young women with false diagnoses of POA/oPOI, and in most extreme cases of POF/POI, for years. Once placed on androgen supplementation, they experienced a "reawakening" of ovaries, with decreasing FSH and improving AMH levels and successful reinitiation of folliculogenesis. Their initial presentation, therefore, represented a previously unknown form of secondary ovarian insufficiency (SOI).

Through androgen production, the adrenals, therefore, can control ovarian function, establishing a functional adrenal-ovarian axis, controlled by adrenal function of the zona reticularis. Considering that the adrenals and ovaries share a common embryonic primordium [38], this relatively recent fining is less surprising than it may appear at first glance. Indeed, even a yet undiscovered ovarian-adrenal feedback would not surprise within this functional adrenal-ovarian axis. AMH may be an intriguing potential candidate since AMH receptors have been reported at very high density in adrenal tissues (at second-highest concentration after GCs), although so far with no defined physiological function [39].

In the diagnostic evaluation of infertile women, adrenals, therefore, now must be viewed as potentially contributing to ovarian function—in the positive or negative.

The Immune System

Endocrine and immune systems are now well recognized to be closely intertwined, and often given the combined synonym "immunoendocrinology" [40]. The ovary is an excellent example, with many important genes for normal ovarian function also essential contributors to homeostasis of the immune system [41].

Like every endocrine organ in the human body, ovaries are subject to autoimmune attacks. But autoimmune oophoritis, a condition characterized by infiltration of the theca by lymphocytic and plasma cell infiltrates, is an exceedingly rare condition, and only seen in association with adrenal autoimmunity (Addison's disease) [42]. That no other autoimmune condition affecting ovaries has so far been described is, indeed, puzzling, especially considering that autoimmunity has been etiologically widely associated with POA/oPOI as well as POF/POI [43]. Considerable evidence also points to a heightened prevalence of autoimmunity in general populations of infertile women [44].

Since both, adrenals and ovaries, poses steroidogenic tissues and derive from a common embryonic primordium [38], some investigators speculated that they may share (possibly among steroidogenic enzymes) epitopes that may be targets of shared autoimmune attacks. But even in cases of autoimmune oophoritis, which is so closely associated with Addison's disease [42], no such common epitopes have so far been discovered. We, therefore, shifted attention to a completely new hypothesis, which suggests that the widely observed autoimmunity in association with POA/oPOS and POF/POI may not be against ovarian but against adrenal epitopes (in the zona reticularis), resulting in adrenal insufficiency (of androgen production) and, therefore, in peripheral hypoandrogenism, so widely observed in association with these two conditions [33,34,37].

Although approximately half of a woman's androgens are of adrenal origin produced in the zona reticularis, current definitions of adrenal insufficiency (Addison's disease), do not include insufficiency of the zona reticularis. Only insufficiencies of the two zonae producing glucocorticoids and mineralocorticoids are currently part of the definition of Addison's disease [45]. Yet, as noted in the preceding section, adrenal hypoandrogenism can significantly impact ovarian function. Observed high prevalence of autoimmune abnormalities, especially autoimmune thyroid disease, in association with adrenal hypoandrogenism [35,43,44], moreover, raises the specter that adrenal hypoandrogenism may also be autoimmune in etiology.

All of these observations support the hypothesis that evidence of systemic autoimmune activity in association with POA/oPOI and POF/POI relates to antiadrenal rather than antiovarian autoimmune responses, and that the long-observed association of systemic autoimmunity with POA/oPOI and POF/POI [43] may, therefore, be indirect. Autoimmune adrenal hypoandrogenism may be causing SOI by adversely affecting normal follicle maturation at small growing follicle stages [37]. This hypothesis would also explain why decade-long attempts to discover antiovarian autoimmunity in association with POA/oPOI and POF/POI have failed.

This hypothesis is also supported by the observation that hypoandrogenic women with POA/oPOI and older women with LOF/LFOR demonstrate mild degrees of hypocortisolemia [33,34,46–48]. With the glucocorticoid cortisol produced in the zona fasciculata, located adjacent to the most inner zona reticularis, this observation suggests that these two zonae are not completely independent (both are also responsive to ACTH). Indeed, since in humans, the zona reticularis contains 17 alpha-hydroxylase (in rodents it does not), pregnenolone in humans is converted in adrenals to cortisol. Adrenal hypoandrogenic infertile women, therefore, may also exhibit insufficiencies of glucocorticoid production and should be accordingly investigated if clinical suspicion so warrants [46–48]. These observations also again raise the question why insufficiency of the zona reticularis is not considered in the definition of adrenal insufficiency [48,49].

Under this hypothesis, autoimmune-induced adrenal hypoandrogenism, therefore, would be the cause of a distinctly different, and previously unknown form of SOI, with not only distinctively different etiology and treatment from POF/POI but also with much better infertility treatment prognosis. The principal reason is that women with POF/POI, likely, exhibit only extremely low OR, while women with SOI, likely, still have largely unaffected ovaries. Chances of resuscitation of OR, therefore, are much better in women with SOI.

HOW THE OVARY CONTROLS TREATMENT SUCCESS IN IVF

Ovaries affect IVF outcomes through the quantity and quality of oocytes produced in treatment cycles. Although both, quantity and quality of oocytes, usually run in parallel, exceptions to this rule have led to better understanding of ovarian functions in IVF. For example, a study investigating correlations between FSH and AMH levels, produced the surprising finding that among different combinations of high, normal, and low levels of these two hormones, the high/high combination produced by far best pregnancy and live birth rates [50]. As women age, FSH and AMH levels, in principle, go into opposite directions. This finding, therefore, was a surprise, and went for a number of years unexplained, as high-FSH/high-AMH as treatment goals appeared contradictory.

Only once a hypoandrogenic PCOS-like ovarian phenotype was described [35], characterized by high-AMH but hypo- rather than the hyperandrogenism that usually characterizes PCOS, was the puzzle at least partially resolved: as it turned out, at first presentation, the high-AMH/high-FSH group represented mostly women with this hypoandrogenic PCOS-like phenotype prior to DHEA supplementation. With supplementation, they turned into high-FSH/high-AMH phenotypes because androgen supplementation improved their FOR and, therefore, their AMH levels, oocyte numbers and quality and, ultimately, clinical pregnancy and live birth rates. The puzzle surrounding the initial study [50], thereby, was finally resolved.

Quantity and Quality of Oocytes

As women age, oocyte yields assume ever greater importance because oocyte efficiency in establishing pregnancy declines. In clinical IVF practice, this has led to transfer of increasing embryo numbers with advancing age in attempts to compensate for declining implantation and pregnancy rates per embryo, as also recommended by periodically updated ASRM guidelines [20].

Fig. 4A and B further demonstrates that at all ages clinical pregnancy and live birth rates almost linearly increase with increasing embryo production in IVF cycles. As at each age similar numbers of embryos were transferred, observed improvements in clinical pregnancy and live birth rates did not only relate to numbers of embryos transferred but also continued with extra cryopreserved embryo numbers. In other words, the number of embryos produced in an IVF cycle not only reflects quantity, likely, but also quality of oocytes and embryos produced in that cycle [14].

As already noted, with few exceptions, quantity and quality of oocytes usually correlate. AMH is, therefore, widely perceived to reflect not only likely oocyte numbers but also oocyte and, ultimately, embryo quality. Produced by GCs, which reflect an essential component of the ovarian microenvironment in which oocytes mature, the ability of AMH to represent the growing follicle pool quantitatively as well as qualitatively, should, therefore, not surprise.

But GCs do not exist in isolation, and their normal proliferation as part of follicular maturation is, especially at small growing follicle stages between primary follicle and small preantral follicle stages, depending on synergism between FSH and testosterone [31,32]. When androgen levels in ovarian microenvironments, in which small growing follicles mature, are inadequate, follicle growth and maturation will slowdown, GC mass will decline, nutritional support for developing oocytes will diminish, and oocyte quantity and quality at time of retrieval will be poor. Abnormally low androgen (i.e., testosterone) levels in the ovarian microenvironments, especially in relative early stages of hypoandrogenism may, therefore, clinically only manifest as poor oocyte and embryo quality, while impairment in oocyte numbers may become apparent only later, at more severe stages of hypoandrogenism.

What Constitutes Oocyte Quality

Oocyte quality overwhelmingly determines embryo quality. One, therefore, would expect considerable attention given to oocyte assessments after egg retrievals. Although most IVF laboratories do assess oocyte quality, these assessments, quite surprisingly, primarily only concentrate on maturity stages of oocytes. Detailed morphological assessments are usually only reserved for embryos.

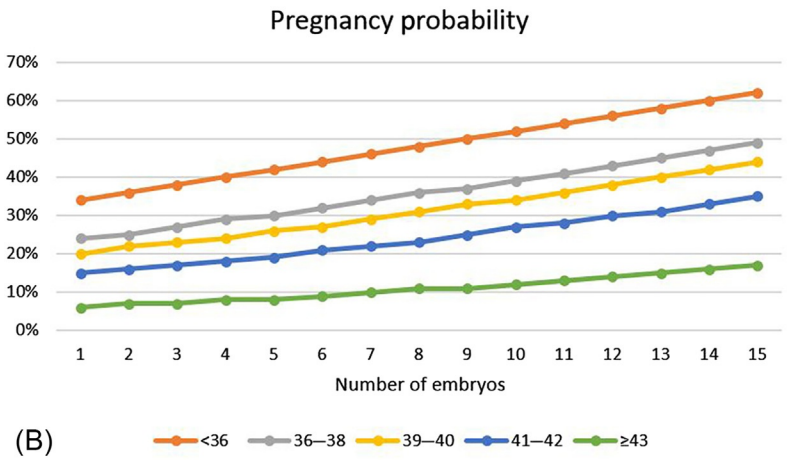
Morphologically

Detailed morphological assessments of embryos have been practice for decades. In recent years, a new industry has arisen from this concept, producing closed incubation systems with time-lapse imaging in attempts to elucidate morphological criteria predictive of implantation chances for embryos. To the surprise of many, these systems, however, have been unable to help in selecting "best" embryos [51,52].

Better understanding of the basic biology of follicle/oocyte maturation should, however, have foreseen that time-lapse imaging would not improve embryo selection over standard manual embryological assessments. In view of the importance of egg quality in determining embryo quality, and given the many weeks to months of folliculogenesis during which egg quality is determined, it appears rather obvious that most of an embryo's ultimate quality is predetermined at very early stages of

Clinical pregnancy rates					
Embryos	Ages				
	<36	36–38	39–40	41–42	≥43
1	34%	24%	20%	15%	6%
2	36%	25%	22%	16%	7%
3	38%	27%	23%	17%	7%
4	40%	29%	24%	18%	8%
5	42%	30%	26%	19%	8%
6	44%	32%	27%	21%	9%
7	46%	34%	29%	22%	10%
8	48%	36%	31%	23%	11%
9	50%	37%	33%	25%	11%
10	52%	39%	34%	27%	12%
11	54%	41%	36%	28%	13%
12	56%	43%	38%	30%	14%
13	58%	45%	40%	31%	15%
14	60%	47%	42%	33%	16%
15	62%	49%	44%	35%	17%

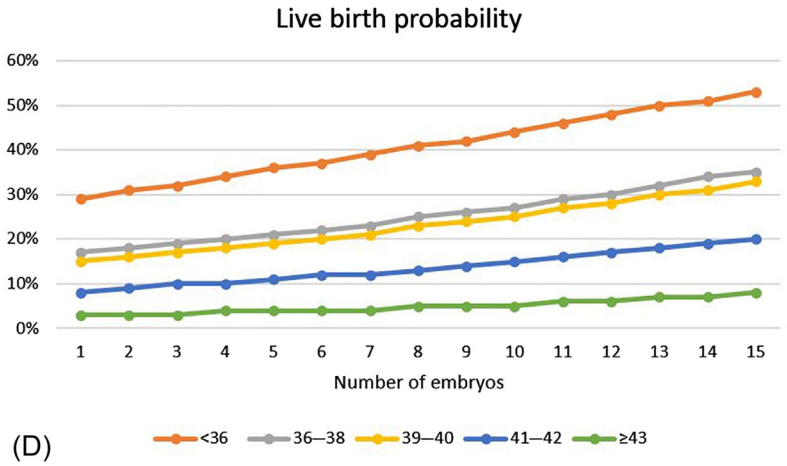
(A)



(B)

Live birth rates					
Embryos	Ages				
	<36	36–38	39–40	41–42	≥43
1	29%	17%	15%	8%	3%
2	31%	18%	16%	9%	3%
3	32%	19%	17%	10%	3%
4	34%	20%	18%	10%	4%
5	36%	21%	19%	11%	4%
6	37%	22%	20%	12%	4%
7	39%	23%	21%	12%	4%
8	41%	25%	23%	13%	5%
9	42%	26%	24%	14%	5%
10	44%	27%	25%	15%	5%
11	46%	29%	27%	16%	6%
12	48%	30%	28%	17%	6%
13	50%	32%	30%	18%	7%
14	51%	34%	31%	19%	7%
15	53%	35%	33%	20%	8%

(C)



(D)

FIG. 4 Clinical pregnancy and live birth rates following IVF at different ages, based on number of transferrable embryos. This figure demonstrates clinical pregnancy (A and B) and live birth rates (C and D) at the Center for Human Reproduction at different ages, dependent on transferrable embryos in a single cycle cohort. The study demonstrates an almost linear relationship between number of embryos available and IVF outcomes, even though embryo numbers transferred in each age group were similar, independent of embryo number available for transfer, suggesting that embryo numbers also, indirectly, reflect embryo quality. *Background colors* of the individual fields reflect good *(blue)* intermediate *(white)* and good prognoses *(yellow). From Gleicher N, Kushnir VA, Sen A, Darmon SK, Weghofer A, Wu YG, et al. Definition by FSH, AMH and embryo numbers of good-, intermediate- and poor-prognosis patients suggests previously unknown IVF outcome-determining factor associated with AMH. J Transl Med. 2016;14(1):172, with permission.*

follicle/oocyte maturation. Oocyte quality assessments after retrieval, in our opinion, therefore, deserve more attention than they currently receive. A recent study confirmed this by demonstrating that morphological features of mature MII oocytes were superior to standard embryo grading in predicting the chance implantation of high-quality embryos that arose from those oocytes [53].

This study was the product of our center's weekly IVF cycle outcome conference, where clinicians and embryologists in a team approach reassess unsuccessful IVF cycles in detail. These weekly analyses led to the recognition that unsuccessful IVF cycles frequently demonstrated discrepancies between morphological assessments of oocytes and embryos. We were especially struck by how often we saw cycles where embryo quality was rated relatively high but initial oocyte quality was not. In many of these unsuccessful cycles, oocyte grading, therefore, appeared to be the better predictor of cycle outcome. Embryo quality may, therefore, at times be misleading if, upstream, the underlying oocyte quality was

poor. Fig. 5 demonstrates the morphological criteria used in this study. The single most important adverse predictor of poor IVF cycle outcome was small oocyte size.

This study, therefore, supported the obvious biological reality that the oocyte predetermines the biggest part of an embryo's implantation potential far upstream from fertilization and preimplantation embryo development stages, where routine embryo assessments are taking place in IVF laboratories. Basic biology, therefore, teaches us that, as principal product of ovarian function, oocytes are the most important building block of IVF outcomes and, therefore, clinically deserve more attention than they currently receive.

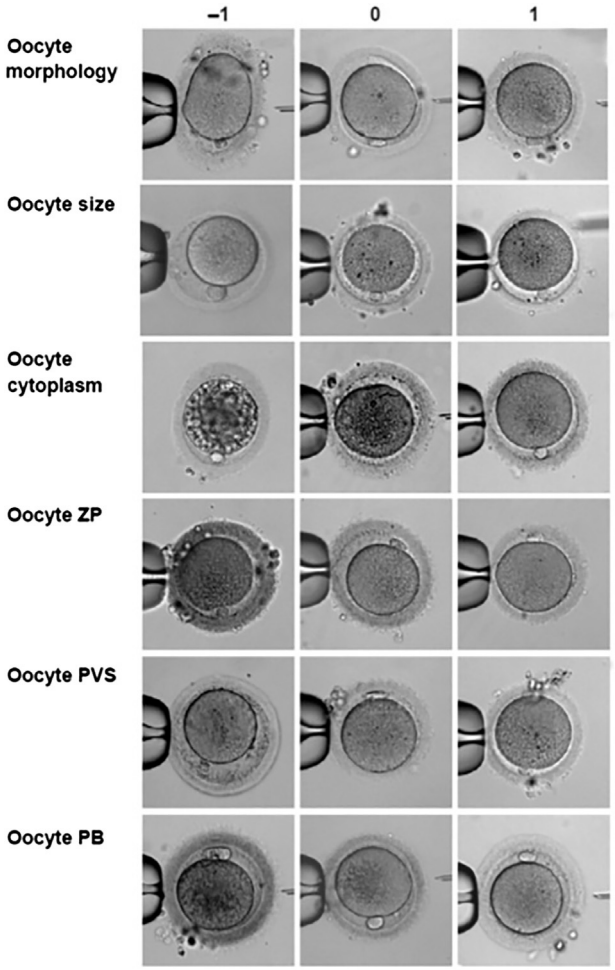


FIG. 5 Example photographs of oocyte scoring system. The figure represents the oocyte grading system for the six morphological characteristics analyzed in this project: morphology, size, cytoplasm, zona pellucida (ZP), perivitilin space (PVS), and polar body (PB). For each oocyte, each single characteristic was graded as worst (−1), average (0), or best (1), creating a total oocyte score (TOS) by adding up individual parameter assessments. *From Lazzaroni-Tealdi E, Barad DH, Albertini DF, Yu Y, Kushnir VA, Russell H, et al. Oocyte scoring enhances embryo-scoring in predicting pregnancy chances with IVF where it counts most. PLoS ONE 2015;2;10(12):e0143632, with permission.*

Chromosomally

During fertilization, sperm enters the oocyte's microenvironment. The oocyte initiates its second meiotic division and releases a polar body, containing a haploid set of chromosomes. This polar body, thus, inversely represents the chromosomal status of the oocyte after its first meiotic division. Yuri Verlinsky and coworkers over 20 years ago recognized that this polar body offers an unique opportunity to potentially assess the chromosomal make up of embryos, as most chromosomal abnormalities in pregnancies were believed to be caused by errors in meiosis. A small minority of such errors of paternal origin, would, after meiosis II, be detectable through analysis of the second polar body [54].

This represented the initiation of the preimplantation genetic screening (PGS) hypothesis, which suggested that elimination of aneuploid embryos prior to replacement into the uterus would increase implantation rates of remaining euploid embryos in a cycle cohort, improve pregnancy and live birth rates and, likely, reduce miscarriages.

Had IVF stayed with Verlinsky's original vision of PGS via polar body biopsies, PGS over almost 20 years of clinical utilization might have avoided at least some of the controversy it has generated over three generations of this test by failing to improve IVF outcomes. Since meiotic errors are usually present in all of an embryo's cells, their diagnosis is significantly more accurate and clinically relevant than diagnosis of mitotic chromosomal errors which, often, have only a mosaic (clonal) distribution and, therefore, can easily lead to false-negative and, especially, many false-positive diagnoses.

Polar body biopsies, however, proved technically too challenging for wide-scale clinical use. The PGS laboratory community, therefore, largely rejected polar body biopsy and, instead, turned to cleavage-stage blastomere biopsies (on day 3) in the first widely used clinical incarnation of PGS (PGS 1.0), and to trophectoderm biopsy at blastocyst stage in the test's second incarnation (PGS 2.0). This opened the test up to large numbers of inaccurate and, often, outright incorrect test results, mostly caused by misdiagnoses of mosaic embryos due to mitotic chromosomal errors [55]. As is now better understood, meiotic and mitotic chromosomal abnormalities at preimplantation stages of human embryos, therefore, have quite different prognostic relevance.

A first ever prospective study of PGS via polar body biopsy, the so-called ESTEEM trial, organized by the European Society for Human Reproduction and Embryology (ESHRE), recently reported first results at the 2017 Annual ESHRE Conference in Geneva, Switzerland [56]. Considering our improving understanding of why PGS in its various incarnations so far has failed to improve IVF outcomes, it did not surprise that this trial also failed to detect outcome improvements in live birth rates. The

study, however, at least demonstrated no harm to IVF outcomes, although that may have been the case because poor prognosis patients were mostly excluded from the study.

After almost a decade of clinical utilization, PGS 1.0 was, finally, formally declared ineffective in improving IVF outcomes less than a decade ago [57–59] but was quickly replaced by PGS 2.0. A single trophectoderm biopsy of five to six cells may, however, easily miss relatively small mitotic clones. On the opposite extreme, accidentally biopsying a small clone, may erroneously lead to the conclusion that the whole embryo is aneuploid. Such false-positive diagnoses turned out to be more frequent than false-negatives, and it increasingly became obvious that single trophectoderm biopsies are, simply, unable to reliably determine the constituency of the whole trophectoderm [60].

Trophectoderm, moreover, reflects the placental cell lineage, while only the inner cell mass (ICM) lineage determines the fetus. Both do not always match in respective chromosomal analyses [61]. Finally, recently published mouse data suggest that, especially in the ICM, embryos have a strong innate ability to self-correct by eliminating abnormal clones even downstream from blastocyst stage [62]. This observation, alone, if confirmed in human embryos, would render any blastocyst-stage embryo biopsy futile.

All of this has relevance to current IVF practice, where in the United States in 2016 approximately 20% of all fresh IVF cycles included PGS [63]. Some of the nation's leading IVF centers, indeed, now mandate PGS in association with IVF. Following a radical revamp in the late summer of 2016 as to how PGS is recommended to be performed and reported out by laboratories (we, therefore, now use the acronym PGS 3.0) [64], the procedure was also renamed by some with the acronym preimplantation genetic testing for aneuploidy (PGT-A).

This latest form of PGS finally acknowledges that trophectoderm of blastocyst-stage embryos to a high degree (and, possibly in virtually all cases) is mosaic. Moreover, a majority of aneuploidies detected at that stage are mitotic, possibly self-correcting and/or clinically irrelevant because they are segregated to the future placenta. The placenta for decades has been known to contain at term isolated aneuploid cell islands in, otherwise, completely normal euploid nonmosaic pregnancies.

Until the July 2016 announcement of PGS 3.0/PGT-A [64], PGS laboratories for almost two decades, uniformly, defined embryos only as either euploid or aneuploid. Every aneuploidy detected, whether meiotic or mitotic, therefore, resulted in exclusion from transfer and disposal of embryos. With the announcement of PGS 3.0/PGT-A, embryos are now reported as either euploid, mosaic, or aneuploid, and patients and clinicians are afforded the choice of transferring mosaic embryos, which until recently universally used to be discarded. A series of reports of such transfers from a number of IVF centers around the world suggested surprisingly robust clinical pregnancy and live birth rates and equally surprisingly low miscarriage rates [65–68]. The most recent and largest report from an international consortium of centers and PGS laboratories, reported a 41% clinical pregnancy rate overall and a 50% clinical pregnancy rate from transfer of single monosomies and trisomies (i.e., equal to the chance of a coin flip). Even complex chromosomal abnormalities, still, resulted in 10% clinical pregnancies [69].

Above noted recently reported ESTEEM trial [56] and these remarkable transfer outcomes of aneuploid/mosaic embryos, therefore, strongly support those voices who have been claiming that the hypothesis of PGS, as good as it sounded at initial presentation, for biological reasons appears deeply flawed, is incapable of improving IVF outcomes and, in at least selected patient groups (primarily women with LOR/LFOR), will actually reduce pregnancy and live birth chances [70,71].

Mitotic aneuploid clones at blastocyst stage have recently attracted increasing attention because early human embryos demonstrate increased expression of gene products favoring cell progression, while lacking expression of cell-cycle checkpoint genes [72]. As has become apparent in cancer, such a constellation favors genetic instability and increased mitotic errors. Preimplantation embryos, thus, like invasive cancer cells, appear physiologically predisposed toward mitotic errors, a finding giving rise to the hypothesis that trophectoderm mosaicism may have a physiological function in promoting trophoblast invasiveness, that is, implantation of the blastocyst-stage embryo.

In metastatic cancer, aneuploid cells not only lead to invasiveness [73], but also subvert the host's immune response to invasiveness [74]. One, therefore, can further hypothesize that aneuploidy of trophectoderm in very early pregnancy may facilitate induction of tolerance for the implanting semiallogeneic embryo [75].

AFFECTING OVARIAN PERFORMANCE

As already noted earlier, the initiation of modern infertility care can be defined by the introduction of gonadotropin treatments in the mid-1950s to early 1960s through the work of primarily Gemzell [28] and Lunenfeld [29]. This treatment is based on growing follicles acquiring gonadotropin sensitivity only during the last 2 weeks of folliculogenesis (in contrast to only a minor degree of FSH responsiveness at earlier stages of follicle maturation).

During a large majority of spontaneous natural cycles, one single dominant follicle evolves, with all other follicles within the monthly follicle cohort degenerating

and undergoing apoptosis. Exposing this cohort of large growing follicles to external gonadotropin supplementation, permits rescue of additional follicles, and the natural mono-follicular becomes a poly-follicular response, improving pregnancy chances but also increasing the risk of multiple births.

Original attempts at IVF by Steptoe and Edwards used natural cycles. Early IVF successes were obtained by obtaining poly-follicular responses through the antiestrogen clomiphene citrate [76]. IVF, however, became a successful clinical treatment only once Howard and Georgianna Jones at the Norfolk IVF program (now the Jones Institute for Reproductive Medicine at Eastern Virginia Medical School) introduced gonadotropin stimulation to IVF [77].

Gonadotropin stimulation has, ever since been the principle ovarian stimulation protocol for IVF. Over the last decade, quite a number of alternatives to standard gonadotropin stimulation have been proposed, although all affect only the gonadotropin-sensitive last 2 weeks of follicle maturation, whether by directly stimulating the ovaries with gonadotropins, by inducing endogenous FSH production via the antiestrogen clomiphene citrate or by aromatase inhibition through drugs like letrozole (Femara).

To discuss individual ovarian stimulation protocols in detail would exceed the framework of this chapter. The following section, however, offers brief descriptions of most frequently utilized protocols. An important point this chapter wishes to emphasize is that, like other areas of medicine, infertility treatments have entered a phase of "personalized medicine," where ovarian stimulation protocols can no longer be universal but have to be individualized.

While young women with normal OR may still be appropriately treated with uniform protocols, older patients and younger women with LOR/LFOR, if best results are to be obtained, must be individualized. Such individualization starts even before initiation of COH because in complex patients, ovaries often need to be "prepared" weeks to months prior to initiation of COH. Ovarian stimulation not only requires individualization of medication dosages but also of length of ovarian stimulation since, with advancing female age and in some women with POA/oPOI, intrafollicular physiological processes speedup [17].

Interventions Into the Gonadotropin Sensitive Stage of Folliculogenesis

We here will briefly summarize how ovaries can be medically prepared to yield oocytes in controlled fashion. To obtain multiple oocytes in a single cycle, has been the basis of modern fertility treatments over the last 50–60 years, first in association with intrauterine inseminations and later in association with IVF. These efforts involving ovarian stimulation, called COH, however, affected only the last 2 weeks of folliculogenesis—the gonadotropin-sensitive stage of follicle maturation. This is important to reemphasize because the following subsection will address interventions into earlier stages of follicle maturation.

Natural Cycle IVF

As already noted, IVF was first explored in natural cycles. Paradoxically, the concept of performing IVF in natural cycles has again gained some following over the last decade. This is somewhat surprising since, after female age, numbers of oocytes retrieved and transferrable embryos are the best predictors of IVF success [14]. Natural cycles produce only one oocyte in approximately 60% of cycle starts. Pregnancy rates in general populations, with reference cycle start, are only in single digits [78]. A more recent study suggested that in youngest patients (<35 years), the ongoing clinical pregnancy rate may be as high as 10.6%; but as low as 3.0% in women above age 40 [79].

Although the worst candidates for natural cycle IVF (and single embryo transfer) [79], older women or women who for other reasons suffer from LOR/LFOR are, paradoxically, the primary patient population treated with natural cycle IVF [6]. Better IVF outcomes in young good prognosis patients, who in contrast produce better quality eggs and embryos, should, therefore, not surprise, even if they end up with only a single embryo for transfer.

Examples of the negative consequences of large-scale single embryo transfers are Japan's national IVF outcome data and those of Australia and New Zealand (both countries report combined), two regions of the world where single embryo transfers have dominated in recent years. Japan over the last decade experienced plummeting live birth rates by two-thirds (Fig. 6A). This decline was time wise closely associated with the nationwide increase in utilization of the Kato protocol, characterized by expanded use of natural cycles and mild stimulations of ovaries [80]. Concomitantly, IVF cycle starts tripled in Japan over the same time period. The country, thus, tripled IVF cycle starts—just to maintain their national live birth rate from IVF. Australia and New Zealand over the last few years also aggressively increased elective single embryo transfer (eSET) cycles, and demonstrated similar, although less pronounced, declines in live births and concomitant increases in cycle starts (Fig. 6B) [22,81].

Mild Stimulation

Mild ovarian stimulations have also found a significant following over the last decade, even though outcome data uniformly demonstrate significantly lower

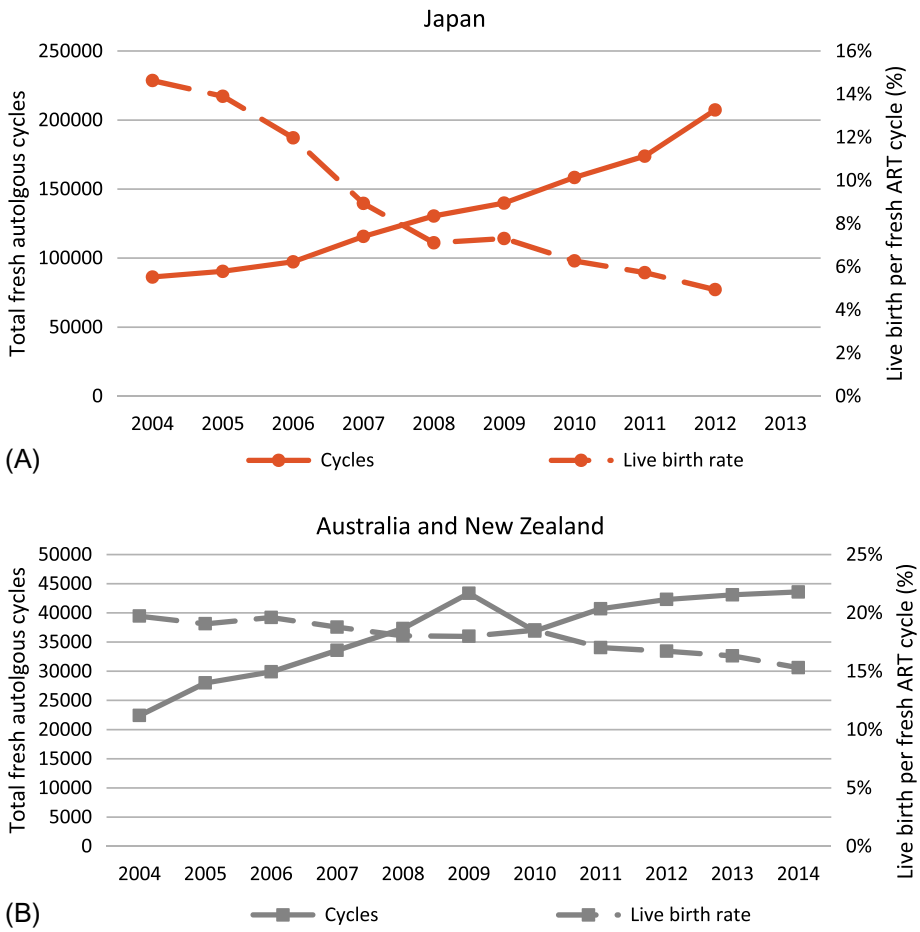


FIG. 6 National live birth rates and fresh IVF cycle utilization of Japan and Australia/New Zealand (combined) over the last 10 years. (A) Japanese live birth rates over the last 10 years plummeted from approximately 15%–5% with reference point cycle start. Concomitantly, the country's fresh IVF cycle starts tripled. These developments coincided with the so-called Kato protocol becoming the dominant protocol in Japanese IVF centers, involving natural cycles or mildly enhanced natural cycles and elective single embryo transfer (eSET) [80]. Australia and New Zealand (B) demonstrate a similar, although somewhat less pronounced, picture, based on rapid increases in eSET utilization over those years [22,81]. *Modified with permission from Gleicher N, Kushnir VA, Barad DH. Commoditization and industrialization of IVF is responsible for worldwide declining IVF birth rates. 2018 [submitted for publication].*

pregnancy and live birth rates with mild stimulation protocols than standard ovarian stimulations. This is even acknowledged by proponents of mild stimulation [82]. Reflecting voluntary reductions in oocyte yields over standard COH, the concept makes physiologically little sense since, as noted before, oocyte (and embryo) yields are prognostic of IVF outcomes.

Mild stimulation gained popularity primarily because practitioners presented it as more "natural" and "patient-friendly" than regular COH. A few studies also supported this perception by claiming that milder stimulation produced less aneuploidy in oocytes and, therefore, better egg quality [83]. Later studies, however, refuted these findings and demonstrated that more aggressive stimulation (i.e., increasing gonadotropin dosages) produced more transferrable embryos [84,85], a statistical parameter always directly associated with pregnancy and live birth rates. Like natural cycle IVF, mild stimulations also heavily contributed under the Kato protocol [82] to above-described astonishingly poor Japanese live birth rates over the last decade [22,81].

We consider mild ovarian stimulation only indicated in avoiding OHSS. Only young women with PCOS should, therefore, automatically be designated to mild stimulation.

Some authors suggested "ideal" oocyte number to be strived for by adjusting gonadotropin dosages accordingly. We, however, question such suggestions since what may appear "ideal" at one age or with a specific OR, my not represent "best" outcomes at different ages or with different OR. Advanced female age and LOR/LFOR are associated with declining oocyte quality and quantity. Age and OR, therefore, will obviously affect what would represent "best" oocyte numbers.

Standard Stimulation Protocols

Table 1 summarizes ovarian stimulation protocols, and the appropriate patient populations they would serve best. Most frequently utilized protocols are few: the so-called long-agonist protocol, especially in younger women with normal OR who can well tolerate the

TABLE 1 Best Ovarian Stimulation Protocols for Different Patient Populations

Cycle protocol	Best patient population
Natural cycle	Young patient with normal ovarian reserve
Mild stimulation	PCOS
Standard stimulations	
Long agonists	Young patients with normal ovarian reserve
Microdose agonists/flair	Above age 40 or low ovarian reserve for other reasons
Antagonists	Patients valuing convenience over outcomes
Niche protocols	
Clomiphene citrate	In combination with gonadotropins by some used in older women
Aromatase inhibitor	Women with history of ER+ breast cancers
Double stimulation	Women with small oocyte yields

suppressive effects of a longer-term administered gonadotropin releasing hormone agonist on ovaries, likely offers best pregnancy and live birth rates for a large majority of IVF cycles. The so-called microdose GnRH agonist protocol, also frequently called the flair-protocol, first proposed by Surrey et al. [16], we, still, consider the best primary protocol for older women above age 40 and younger women with LOR/LFOR, suffering from POA/oPOI.

In many IVF centers, more so in Europe than the United States, the GnRH antagonist protocol has achieved considerable popularity over the last decade. We, however, describe this protocol to patients as IVF's "convenience protocol," because of its patient—and doctor-friendliness in shortening cycles, decreasing overall required gonadotropin dosages and for allowing centers to run weekend-free schedules. Based on our interpretation of the literature and our center's own clinical experiences, we, however, concluded that this protocol mildly reduces clinical pregnancy and live birth rates in routine patient populations and, even more so, in poor prognosis patients. Considering our center's highly unfavorable patient population, we, therefore, hardly ever use this stimulation protocol.

Since our center now performs oocyte retrieval for older women with LOR/LFOR at smaller lead follicle sizes (16–18 mm or even smaller) than younger women [17], the need to prevent premature ovulation in such patients is greatly diminished. Utilization of all agonist protocols, therefore, has also greatly diminished over the last few years.

Niche Protocols

Under this rubric, we list in Table 1 the utilization of clomiphene citrate, aromatase inhibitors, and the concept of double stimulation. Clomiphene citrate is the longest available fertility medication and by many, still, considered the preferred first-line medication in treating young infertile women. As noted earlier, it was the medication used to convert spontaneous mono-follicular cycles to poly-follicular cycles during the very early days of IVF [76], only to be quickly replaced by ovarian stimulation with gonadotropins [77].

The reasons were manifold: clomiphene citrate and aromatase inhibitors are practically the only orally administered fertility medications. Although gonadotropins and GnRH agonists/antagonists are subcutaneously injected, clomiphene fell quickly out of favor. The primary reason was poorer efficiency in rescuing follicles from degeneration and apoptosis and, therefore, smaller oocyte production than is achieved with gonadotropin stimulation. In addition, early IVF experiences established better implantation rates from embryos after gonadotropin stimulation, although whether this observation was a reflection of poorer egg/embryo quality or negative effects of clomiphene citrate on the endometrium was never resolved. Clomiphene clearly thins out the luteal endometrium. In addition, the drug in many women causes significant mood swings. It, therefore, is frequently severely disliked by patients.

The popularity of clomiphene citrate as a niche player was in recent years partially resurrected by the previously noted Kato protocol [80] which, unless utilizing natural cycles, combined 5 days of clomiphene stimulation with minimal dosages of gonadotropins (75 IU) on alternating days. As previously noted, this protocol appears to be the principal reason why Japan has been experiencing the by far lowest live birth rates in the world ([22,81] and Fig. 6). Claims of better outcomes with the Kato protocol in the United States [86], were also refuted [87]. In more recent years, a combined clomiphene/gonadotropin protocol, although with much higher gonadotropin dosages, has been proposed for use in older women with LOR/LFOR. Its ultimate efficacy in this patient population remains to be established.

To a degree, whereas many fertility centers have completely abandoned clomiphene, utilization of aromatase inhibitors has increased over the last decade. Whether one or the other results in better pregnancy rates as a first-line fertility drug, has remained controversial. In IVF cycles, aromatase inhibitors are, however, only rarely used. Their primary indication is in young women diagnosed with estrogen receptor-positive (ER+, breast) malignancies who, prior to initiation of chemotherapy, still wish to cryopreserve oocytes.

Studies in recent years demonstrated that ovarian stimulation starts do not have to be restricted to traditional cycle start dates on second/third days of follicular phase or, for long GnRH agonist protocols, to days 23/24 in the luteal phase. Stimulations, indeed, can be initiated at practically almost all times [88]. From this observation evolved the concept of "double stimulation" within one cycle-month, with the principal goal of maximizing oocyte yields within a short time period, like required by cancer patients prior to chemotherapy. Under this concept, a second ovarian stimulation can be initiated immediately after retrieval of a first such stimulation, resulting in approximately doubling of oocyte yields within a single month [89].

Although ovarian stimulation protocols, therefore, quite obviously can greatly differ, all here described protocols affect follicle maturation in only the last 2 weeks of folliculogenesis. Considering that the whole period of follicle maturation following recruitment out of resting primordial follicle stages takes months, it appears obvious that by the time follicles reach the gonadotropin-sensitive stage, their fates and, therefore, likely quality of oocytes, has been largely determined. If further progress is to be made in improving oocyte numbers and quality, pharmacological interventions into folliculogenesis, therefore, have to be made into earlier stages of follicle development. This will be further addressed in the next subsection.

Interventions Into Earlier Stages of Folliculogenesis

Androgen Supplementation

Currently, only one example exists for successful pharmacological intervention into early stages of folliculogenesis, and that involves androgen supplementation in women with low peripheral androgen levels. The underlying pathophysiology is declining testosterone with advancing female age, but also with all forms of LOR/LFOR [31–34] and, therefore, insufficient testosterone concentrations in the ovarian microenvironment, in which follicles mature after recruitment.

In a number of different animal models [31,36], the importance of appropriate androgen levels in ovaries has been well established over the last decade, with classical initial studies reported in a mouse androgen-receptor knockout model by Sen et al. [31]. These studies demonstrated how important testosterone was at small growing follicle stages between primary and small antral follicle stages for normal quantitative as well as qualitative follicle development. They also demonstrated through knockouts of androgen receptors on GCs and oocytes that it is the GC receptor, which causes most of the damage. Knockout of the androgen receptor on oocytes had only relatively minor negative consequences on reproductive outcomes [31,37].

Concomitantly, clinical studies increasingly reported improved IVF cycle outcomes with androgen supplementation in older women (above age 40) and younger women with POA/oPOI, who almost uniformly prior to supplementation demonstrated low testosterone and, often, high SHBG levels [33–35]. Because properly powered clinical trials of androgen supplementation have not yet been performed (and likely never will), androgen supplementation in reproductive medicine, although increasingly widely utilized around the world, has remained controversial.

Considering that physiology as well as molecular biology of synergism between testosterone and FSH at small growing follicle levels have been well described [37], this skepticism is somewhat surprising but is, likely, the consequence of poorly performed clinical studies. A small number of clinical trials of androgen supplementation were uniformly underpowered, whether they reported IVF outcome improvements or not. Yet, other clinical supplementation studies are uninterpretable because they failed to understand that androgen supplementation primarily benefitted only small growing follicles and, therefore, had to be administered weeks to months ahead of IVF cycle start. A good number of reported trials supplemented androgens only during cycle stimulations and/or for brief periods before cycle starts and, therefore, lacked any physiological logic. Androgen supplementation in association with IVF must be started at least 6–8 weeks before IVF cycle start. In women with LOR/LFOR, our center, therefore, does not initiate IVF cycles unless testosterone and SHBG values have reached desirable ranges. As we discussed in the preceding section on the newly discovered adrenal-ovarian axis, once androgen levels have normalized, IVF outcomes will improve because of improved egg numbers and better egg and embryo quality.

The most active physiological androgen in this process is testosterone [37], acting primarily via the androgen receptor on GCs. GCs, of course, represent the microenvironment of maturing oocytes, which, ultimately largely determine egg quality and fate.

DHEA and DHEAS are the precursor stages for testosterone. Since we initiated androgen supplementation in women with LOR/LFOR over a decade ago, our preference has been supplementation with micronized oral DHEA (25 mg TID) over direct administration of testosterone. After obtaining baseline levels for androgens and SHBG, we maintain the dosage uninterrupted (including ovarian stimulation periods) until a patient conceives or terminates treatment with use of her own eggs. As noted before, desired testosterone levels are difficult to define. We strive for levels above normal midranges for free as well as total testosterone. Total

testosterone has been demonstrated the somewhat better predictor of IVF outcomes in comparison with free testosterone. In addition, the delta increase in testosterone after DHEA supplementation was demonstrated to be predictive of successful pregnancy: the wider the delta between presupplementation levels and levels at IVF cycle start, the better were the chances of pregnancy [33].

As also noted before, hypoandrogenic women usually demonstrate relatively elevated SHBG levels, which normalize as testosterone levels improve. Ideally, we like SHBG to be under 80 nmol/L before IVF cycle start. SHBG, thus, helps in establishing when women's testosterone levels have reached desired range and balance with SHBG. This determination can be complicated because what is considered "normal" can vary greatly between individuals, especially if they are past PCOS patients (the PCOS phenotype improves with advancing age). Increasing evidence suggests that women with PCOS carryover receptor memory for high testosterone levels from younger years. They can be identified by reducing SHBG to normal levels only at higher testosterone than women with normal ovarian phenotype. Since SHBG can also be affected by other hormones (e.g., hyperthyroidism), obesity, and age, interpretations have, however, to be made with caution.

Following DHEA supplementation, testosterone levels in most women will increase quickly into expected ranges. As small minority, especially among women of African descent, will, however, convert DHEA to testosterone only poorly [90]. They, therefore, will have to be supplemented with testosterone directly. This can be done by testosterone patch or testosterone gel. Our preference for DHEA over testosterone supplementation lies in the fact that many organs have the machinery to convert DHEA/DHEAS to testosterone. They do so by drawing only desired amounts of DHEA from the circulation to achieve that particular organ's androgen/testosterone homeostasis. Direct testosterone supplementation, in contrast, floods all organs with identical levels of testosterone, therefore resulting in more side effects.

Our interpretation of the published literature is that androgen supplementation in women with LOR/LFOR significantly improves IVF outcomes [37,90]. This statement should, however, be considered with the understanding that we have conducted most of the early androgen supplementation research in reproductive medicine, and hold a number of US patents claiming outcome benefits from such supplementation in infertile women (see also Conflict of Interest for further details).

Androgen presupplementation prior to IVF, however, offers an excellent example, how during early stages of follicle maturation pharmacological interventions into the ovarian microenvironment improves follicle/oocyte quantity and quality. As we already previously noted in discussing the "CHR concept of ovarian aging," our experience with androgen supplementation in women with LOR/LFOR led us to believe that the aging process affecting the ovarian environment plays an essential role in why older women produce poorer quality eggs and embryos. Like androgen supplementation seemingly has the ability to improve egg and embryos quality, so should innumerable other bioactive compounds which change in expression with advancing female age within the ovarian microenvironment. A more complete reconstitution of "older" ovarian microenvironment should, therefore, offer additional advantages over those over the last decade observed with androgen supplementation.

Reconstitution of a "younger" ovarian microenvironment may not only be possible through supplementation of biologically active substances but, possibly also may involve mechanical interventions. For example, as a byproduct of steady ovulations (an inflammatory process that requires "healing" and, therefore, scar formation), advancing female age is characterized by steady increases in ovarian fibrosis. Evidence has been presented in various bodily microenvironments in recent years that increasing pressure of such fibrotic processes upon cells within microenvironments may affect their function.

It, therefore, is conceivable that releasing such pressure through mechanical interventions (i.e., controlled tissue destruction) may have beneficial effects on ovarian function. in vitro induction of the Hippo pathway in ovarian tissue from women with POF/POI with phosphatase and tensin homolog inhibitors and phosphatidylinositol-3-kinase activators has been reported to induce folliculogenesis [91]. The Hippo pathway is, however, also highly mechanosensitive [92] and, therefore, potentially inducible via mechanical interventions.

HGH Supplementation

HGH has been supplemented in IVF cycles on and off for almost 30 years. Results have been mixed, with some reports supporting effectiveness in improving IVF outcome, while others were unable to demonstrate benefits. Quietly, and not based on any new published data, use of HGH in IVF cycles has in recent years increased. Some centers, indeed, have started using HGH almost routinely, especially in women with LOR/LFOR. This expanded use is, however, not predicated on new outcome information but is anecdotal. It, therefore, does not surprise that currently used supplementation regiments greatly vary in timing as well as amounts of medication.

Almost all IVF centers that supplement patients with HGH, do so only during the length of the ovarian stimulation cycle, at best starting only 1–2 weeks before cycle initiation. Like androgen supplementation, physiological effects of HGH (via IGF-I), however, principally benefit small growing follicle stages. If the purpose of HGH

supplementation is to enhance COH by getting more and better oocytes, administration of HGH during only ovarian stimulation makes little sense. Beneficially affected small growing follicles, like with androgen supplementation, will still need weeks to months to become available to gonadotropin stimulation in an IVF cycle. Our center, therefore, currently conducts a prospectively randomized open-label study of HGH supplementation in women with an extremely low OR/FOR, initiated at least 8-week prior to IVF cycle start.

In contrast to androgen supplementation, we consider HGH, however, not an established treatment option for women with LOR/LFOR. Should HGH supplementation, however, turn out to improve IVF outcomes, then it would represent a second example for successful early pharmacological intervention into follicle maturation.

Timing of Oocyte Retrieval

Timing of oocyte retrieval has, likely been one the most consistent features of IVF cycles between centers: when IVF cycles were stimulated with clomiphene citrate, hCG triggers were given at lead follicle sizes of at least 22 mm. Once gonadotropin stimulations took over, lead follicle sizes at ovulation trigger were reduced to approximately 18–22 mm. Recently, molecular evidence was presented suggesting that in older women above age 43 premature luteinization engulfs growing follicles much earlier than at younger ages because molecular processes speedup with advancing age [17].

Consequently, we started retrieving older women at smaller lead follicle sizes, shaving 1–2 days off cycle stimulation length. Although this resulted in retrieval of slightly more immature oocytes, the number of transferrable embryos actually increased and, with it, also clinical pregnancy rates [17]. Later studies then demonstrated that younger women with LOR/LFOR due to POA/oPOI demonstrated similar accelerations in molecular processes within follicles, and that 16–18 mm in both of these patient groups, likely, represented best lead follicle sizes for hCG triggers [93].

As already noted in the preceding section, earlier egg retrievals at our center increasingly reduced the need of microdose agonist use in attempts to prevent premature ovulation since premature ovulation at such small follicle sizes even in older women is exceedingly rare.

IN VITRO MANAGEMENT OF OOCYTES AND EMBRYOS

Individualization of patient care does not end with egg retrieval but now also continues into the embryology laboratory. For example, as older women or younger patients with LOR/LFOR are retrieved earlier and more immature oocytes are obtained, in vitro maturation rescue of immature oocytes becomes more important. If successful, such rescue improves clinical pregnancy rates [17,94]. Especially MI-stage embryos, therefore, should not be discarded, as significant numbers will mature to MII-embryos with overnight culture [94].

Of even greater importance is individualization of length of embryo culture. Increasingly, IVF centers, as standard for all IVF cycles, have embraced blastocyst-stage embryo culture to days 5/6, and more recently even up to day 7. This practice has its roots in reports from Schoolcraft's group, demonstrating improved implantation rates for embryo transfers at blastocyst stage in comparison with cleavage-stage embryos [95]. Their study, however, was performed in mostly good prognosis patients. Follow-up studies in average prognosis patients, indeed, were unable to confirm their initial report [96]. Blastocyst-stage cultures, therefore, cannot universally be presumed to improve implantation rates in all patient populations. Especially in relatively poor prognosis patients, cumulative pregnancy rates from a single cycle cohort of embryos may actually be better with cleavage-stage transfers [97,98].

Assuming this observation to be correct, it would suggest that, especially in poorer prognosis patients, marginal embryos, which in even good embryology laboratories do not survive to blastocyst stage, if transferred at cleavage stage, may still result in perfectly healthy pregnancies. Our center, therefore, never cultures embryos to blastocyst stage, and always transfers at cleavage stage, unless patients are very young have normal OR and large numbers of excellent day 3 embryos.

CONCLUSIONS AND THE FUTURE

By being the source of oocytes, ovaries are the dominant organs in reproductive success, whether in spontaneous conception or in IVF cycles. We here attempted to outline in its total complexity their role during the IVF process.

In over 30 years of IVF as a routine clinical procedure, and with more than five million live births so-far reported worldwide, the procedure has to be viewed as an almost unprecedented medical success, especially since outcomes have (at least until a few years ago) constantly improved.

It now, however, appears that reproductive medicine stands on the verge of yet another major milestone, as new techniques and technologies are coming on line, which promise truly revolutionary options, including and excluding the ovaries. Probably the soonest to be applied clinically, are laboratory culture techniques that will allow in vitro cultures of primordial follicles to

maturity. Acquiring this ability, would not only completely upend current infertility treatments (and disrupt the pharma industry's current pharmacological interventions for COH), but also would go far beyond that: imagining that a small biopsy of the ovarian capsule would yield hundreds, if not thousands of primordial follicles, which then could be matured in vitro and cryopreserved, opens radically new fertility treatment options for women at all ages, and into advanced ages.

But the future may be even more extremely radical: already successfully done in mice [99], it is only a matter of time until human oocytes and sperm will be produced from induced adult pluripotent peripheral stem cells obtained from skin, mucuous membranes, or even hair follicles. The ovary as source of oocytes would become unnecessary, and oocyte supply would be even more unlimited than with above noted ability to mature primordial follicles in vitro.

And consider the opportunities CRISPR-Cas9 editing will offer in eliminating pathological nDNA and mDNA mutations and, therefore, cure single-gene diseases. Here, too, mouse work has already established the possibilities [100], and even human experimentations have started [101,102].

We hope to have outlined in this chapter the primacy we assign, within the context of continuously improving fertility treatments, to the aging ovary. With, especially in developed countries, ages of women undergoing fertility treatments rapidly increasing, we see no more urgent problem. This trend is further enhanced by IVF in young women with normal OR having become a commodity, with most IVF centers being able to achieve good pregnancy and live birth rates. Consequently, these good prognosis patients quickly conceive after entering fertility treatments, while older and, therefore, more difficult to treat patients often linger over many repeat cycles. Fertility centers, therefore, must be ready not only for aging patient populations but also for patients with increasingly poor prognoses.

Conflict of Interest

N.G. and D.H.B. are coinventors on several pending and already awarded US patents claiming therapeutic benefits from androgen supplementation in women with low functional ovarian reserve and relating to the *FMR1* gene in a diagnostic function in female fertility. Both receive royalties from Fertility Nutraceuticals, LLC, in which N.G. also holds shares. N.G., D.H.B., and V.A.K. also are coinventors on three pending AMH-related patent applications. All authors received research grants, travel funds, and speaker honoraria from Pharma companies, although none in any way related to hear presented materials.

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