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Perspective

Applying an evolutionary perspective to assisted reproductive technologies

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Abstract

Assisted reproductive technologies (ART) are commonly used to address human infertility and to boost livestock production. During ART, procedures such as in vitro fertilization, artificial insemination, and intracytoplasmic sperm injection introduce gametes and embryos to unnatural and potentially stressful conditions that can influence offspring health, often via epigenetic effects. In this perspective we summarize these key risks of ART for embryonic and longer-term offspring fitness, emphasizing the need for experimental research on animal models to determine causal links between ART and offspring fitness across multiple generations. We also highlight how ART can bypass a range of naturally and sexually selected mechanisms that occur in the female reproductive tract and/or via female secretions that ultimately determine which sperm fertilize their eggs. We further argue that this curtailment of female-modulated mechanisms of sperm selection may have important consequences for ART-conceived offspring. We encourage the development of ART methods that better mimic natural processes of sperm selection and embrace the fundamental principles of natural and sexual selection. Ultimately, the aim of this perspective is to encourage dialogue between the fields of evolutionary biology and applied areas of animal and human reproduction.

Keywords: assisted reproduction, cryptic female choice, haploid selection, offspring health, infertility

Significance Statement

Assisted reproductive technologies (ARTs) are routinely used to treat human infertility and boost animal production, but they come with recognized epigenetic risks to offspring. Here, we briefly summarize these risks but also highlight how ARTs can inadvertently bypass a range of naturally and sexually selected processes that define the epigenomes of gametes, determine which sperm will fertilize eggs, and ultimately affect offspring health and fitness. The nexus we seek to achieve in this article is to encourage dialogue between the fields of assisted reproduction and evolutionary biology in order to improve both short-term (fertilization) and long-term (offspring viability, and potentially that of subsequent generations) outcomes of ART.

Introduction

Assisted reproductive technologies (ARTs) are employed routinely in human clinical practice and animal breeding and collectively describe a range of treatments targeted at addressing infertility (e.g. in humans) and boosting livestock production (in agricultural and aquaculture settings). Common ART procedures include in vitro fertilization (IVF), intrauterine insemination, artificial insemination, intracytoplasmic sperm injection (ICSI), and the in vitro culture of gametes and embryos. While the benefits of ART across the human fertility and animal production sectors are clear and unambiguous, the last decade has seen an explosion of studies highlighting the possible deleterious and unforeseen consequences of ART, particularly relating to the health prospects of resultant embryos and offspring.

ART procedures are necessarily invasive and involve exposing gametes and developing embryos to a range of artificial and potentially stressful environmental conditions, including during storage (e.g. cryopreservation), handling, transport, and exposure to unnatural chemically defined media. Many of these procedures are thought to directly alter the epigenomes of gametes and additionally exclude natural processes timed around the critical windows of fertilization and early embryonic development. Collectively, these stages involve major epigenetic reprogramming and genomic imprinting events that generate epigenomic signatures that regulate the expression and repression of specific genes [e.g. Sciorio et al. (1)].

To varying extents, ART procedures also bypass natural (and sexual) selection filters in the female reproductive tract (FRT)

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Box 1. Natural and sexual selection in the context of ART

In this perspective, we refer to both "natural selection" and "sexual selection" in the context of designing ART procedures and diagnostic tools that incorporate biologically realistic fertilization environments. Here, we briefly define these evolutionary processes and provide context for their use in the article.

When we use the term "natural selection" (or "naturally selected processes"), we refer to selection acting on phenotypic variation that impacts an individual's fitness *outside* the context of reproductive competition. For example, a trait that confers a survival advantage to its bearer would be subject to natural selection. By contrast, when we refer to "sexual selection," we are more narrowly focusing on selection that generates variance in reproductive competition (e.g. fertilization success when ejaculates from 2 males compete to fertilize a female's ova; sperm competition) (6). For example, if variation in the composition of female reproductive fluid (FRF) influenced a given male's fertilization success at the expense of his same-sex rival's, phenotypic variation in FRF composition would be subject to sexual selection.

To provide context for this review, a clinician might choose to include FRF during in vitro fertilization to preserve the epigenetic state of sperm from donor males, which in turn might confer a survival advantage to ART offspring. Such interventions would be considered as *naturally selected*, as the process would benefit offspring irrespective of the identity of their fathers. By contrast, the use of FRF to differentiate among ejaculates from competing males (e.g. by filtering out unsuitable sperm or reproductively incompatible sperm, or by ensuring fertilization by superior sperm) would constitute a sexually selected intervention. For example, during in vitro fertilization for animal production, ejaculates from multiple males might be used to boost the likelihood of fertilization biases toward optimal males.

(see Box 1 for examples of naturally and sexually selected processes in the context of ART). Ultimately, these filters are thought to prevent defective, incompatible, or fertilization-incompetent sperm from fertilizing the oocyte(s) (2), but they may also serve to select haploid variants that offer a better "genetic match" to the female (3). ART procedures can also bypass female-modulated processes that regulate key epigenetic events and ultimately influence the survival or health prospects of emergent offspring (4). Bypassing these female-modulated processes has only recently been considered as a potential risk factor associated with ART (5).

The aim of this perspective is to summarize some of the key risks of ART for embryonic and longer-term offspring fitness and highlight the need to consider both naturally and sexually selected mechanisms of sperm selection that influence the health prospects of ART-conceived offspring (see Fig. 1). We focus on (i) the potential for ART to impose genetic and epigenetic changes in gametes and offspring and (ii) the potential risks of bypassing natural (e.g. female-modulated) filters that select high-quality sperm and/or refine patterns of fertilization. We anticipate that future progress in this area will come from applying basic evolutionary principles to the practices of gamete selection and ART protocols. Throughout, we draw on evidence from both human and nonhuman studies across the applied fields of ART and fundamental research on reproductive ecology and evolution.

ART and offspring fitness

When evaluating the risks of ART on offspring health, it is important to separate causal factors associated with the ART treatment itself and the possible role that the factors leading to parental infertility can play (7). For example, lifestyle factors associated with infertility (e.g. smoking, obesity, excessive alcohol usage) are themselves known to affect offspring health via mechanisms of nongenetic inheritance [e.g. Evans et al. (8)]. Similarly, infertility can be associated with parental age, and spontaneously conceived children born to older parents can exhibit many of the same health problems experienced by ART-conceived children (9). Thus, simply revealing an increase in the prevalence of health problems in ART-conceived children does not equate to a causal relationship between these factors (see Box 2).

In this section, we consider potential mechanistic factors that may explain the links between ART and offspring health, highlighting where possible studies that are able to experimentally apportion or infer a causal association between ART and health outcomes in offspring. We commence by considering how ART-related procedures such as cryopreservation may influence the gamete's epigenome. We then consider the broader links between ART and offspring fitness before exploring evidence that ART can inadvertently generate epigenetic alterations in offspring.

ART-altered environments

ART typically exposes gametes to artificial storage conditions, preservation, unnatural storage media, and aging (see bottom of Fig. 1), all of which have the potential to affect gene expression and lead to physiological changes in gametes and embryos via alterations in the gametes' epigenomes. For the purposes of illustration, we focus on cryopreservation, which is used to preserve the structure of intact living cells and is known to alter the genetic and epigenetic structure of gametes in both sexes [reviewed by Estudillo et al. (19)]. During sperm cryopreservation, the stability of messenger RNA and the epigenetic content of spermatozoa are thought to be modulated during the freeze-thaw process [reviewed in Hezavehei et al. (20) and Sciorio et al. (21)]. In experimental work, Jia et al. (22) reported that in mouse zygotes, embryos obtained from cryopreserved sperm had higher relative methylation levels and less transcript abundance of *Tet3*—a gene involved in zygotic DNA demethylation—than those arising from fresh sperm controls, possibly due to delayed fertilization when using cryopreserved sperm. Furthermore, Xu et al. (23) reported that cryopreservation of human and mouse sperm can change the expression of microRNAs (miRNA) and may further compromise fertility rates and embryonic development. In the case of oocytes, studies are limited, but there is evidence that cryopreservation (including use of cryoprotective agents) can induce epigenetic and transcriptomic changes in oocytes and embryos [reviews by Estudillo et al. (19), Sciorio et al. (21), Shadmanesh and Nazari (24)]. In summary, there is accumulating evidence from various studies that the procedures around gamete cryopreservation can induce epigenetic modifications in sperm and oocytes that may have implications for offspring traits.

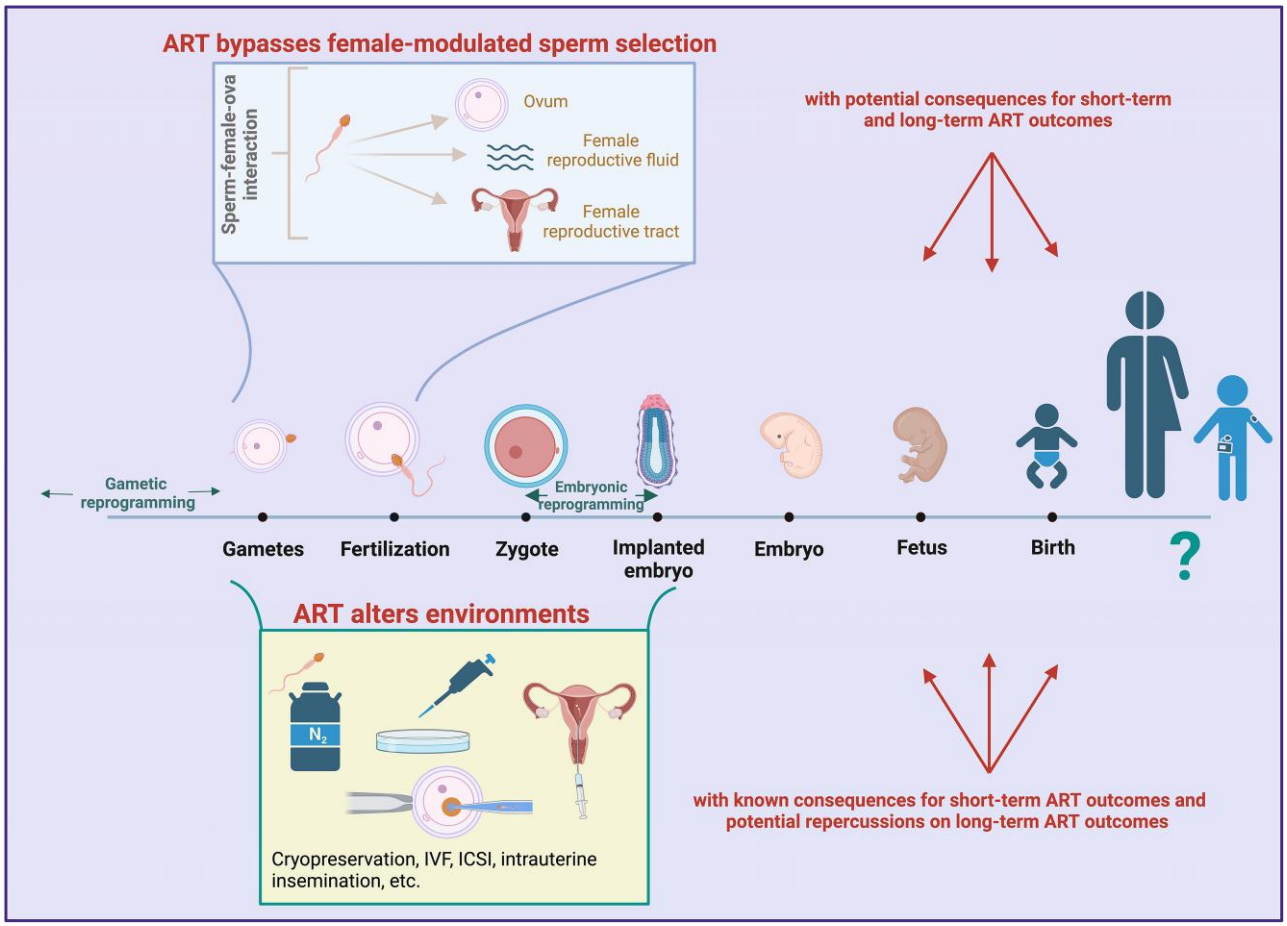

Fig. 1. ARTs can bypass female-modulated sperm selection (top) that ensure that genetically compatible or competent sperm are used for fertilization. ARTs can also introduce a range of novel environmental stressors (bottom) that generate epigenetic modifications in offspring. Failure to design procedures that both mimic natural conditions and mitigate the harmful effect of unnatural environmental conditions during ART can impact the health trajectories of ART offspring and potentially their descendants. Created in BioRender (https://BioRender.com/e43k194).

Box 2. Correlation vs. causal effects

While many of the risks associated with ART, in terms of impacts on early embryonic development and offspring health, have been discussed extensively (see references in the main text), unravelling the causal factors that link ART to reductions in offspring fitness has proved challenging, particularly in human case studies that lack the level of experimental control needed to tease apart causal from correlative effects. In humans, for example, approximately half of the cases of male infertility are associated with genetic effects [see Jiang et al. (10)], and ART processes such as ICSI may inadvertently facilitate the transmission of paternal pathologies to offspring.

Couples undergoing ART are rarely representative of the population at large; they are typically older and generally exhibit underlying (often unknown) etiologies producing infertility or subfertility that may themselves impact offspring health (7, 11, 12). For example, imprinting disorders in ART-conceived children can be attributed, at least partially, to sperm carrying the intrinsic imprinting mutations of their fathers (12). Various imprinting errors may originate due to factors such as abnormal spermatogenesis, which are subsequently transmitted to embryos via ICSI [see Sciorio and El Hajj (13)]. Consequently, for many inherited diseases we must establish whether the association between ART and the ensuing offspring defect is causal or instead attributable to the genetics or pre-existing conditions of couples seeking ART (7, 10).

The links between infertility and sperm DNA damage are well established (12), and sperm DNA damage is known to lead to perinatal (e.g. pregnancy loss after IVF and ICSI) and other offspring problems (14, 15). For example, Xavier et al. (16) highlighted how ART-treated infertility can be attributable to oxidative stress in the male germline, associated with conditions such as age, obesity, or exposure to toxicants. According to this idea, oxidatively damaged spermatozoa may transmit disorders to offspring (including infertility itself) (17), especially if the oocytes' attempts to repair the sperm DNA damage are defective or inefficient (the "defective DNA repair model" leading to paternally derived de novo mutations in the offspring) [see (18)]. Importantly, because procedures such as ICSI bypass selective sieves (e.g. female-modulated selection), they may result in a spurious association between ART and offspring conditions.

Evidence that ART is associated with impaired offspring fitness

It is now widely accepted that parental environmental experiences (e.g. lifestyle, diet, social stress, environmental toxins) can be transmitted across generations via epigenetic modifications) (8, 25, 26). Similarly, ART procedures have the potential to transfer epigenetic marks to offspring, potentially impacting the health and development of offspring across multiple generations.

Evidence for adverse (intergenerational) effects of ART focus mainly on perinatal outcomes, including heightened risk of intrauterine growth restriction, preterm birth, congenital abnormalities, low birth weight and associated mitochondrial genotypes, and a range of maternal disorders (e.g. placental dysfunction, hypersensitive disorders) [see review by Zhang et al. (7)]. In numerous species, suboptimal conditions during ART have been associated with impaired offspring viability and quality, including embryonic stress responses in the form of altered gene expression and/or modification in epigenetic marks established during the early developmental stages [reviewed by Jiang et al. (10), Ramos-Ibeas et al. (27)].

Many complications around ART can also lead to longer-term health risks for children, although these are only now becoming apparent in humans given the relative recent history of ART. For example, ART-conceived offspring have an increased risk for preterm birth [e.g. (28–30)], and preterm human offspring endure greater risks of mental retardation, impaired cardiovascular function, and chronic illness later in life (31–33). ART children also endure heightened risks of contracting noncommunicable diseases such as childhood cancers, asthma, obesity, metabolic syndrome, diabetes, cardiovascular diseases, and neurodevelopmental and psychiatric disorders [reviewed by Zhang et al. (7), Pinborg et al. (34), Li et al. (35)]. Recent studies have also considered the intergenerational reproductive health risks of ART, in which evidence from initial cohorts of ART offspring suggests that men conceived through ICSI produce ejaculates with lower median sperm concentrations, lower total sperm counts, and impaired motility compared with their spontaneously conceived counterparts [(17), see also Crafa et al. (36) for a systematic review and meta-analysis]. However, as we stressed previously (and Box 2), caution is needed in interpreting these results, particularly in human studies in which it is difficult to separate the role of underlying parental infertility from the procedures carried out during ART.

Evidence for epigenetic alterations in ART offspring

ART procedures typically coincide with the period when gametes and embryonic cells undergo epigenetic reprogramming (4, 13). This reprogramming may be sensitive to the environmental variation created by ART. As such, the artificial conditions experienced by gametes and/or embryos during this critical window may lead to epigenetic aberrations in the resultant offspring.

Procedures associated with ARTs have been implicated in disrupting genomic imprinting (paternal or maternal gene silencing through DNA methylation). For example, there is evidence that some imprinting disorders (e.g. Beckwith-Wiedemann syndrome, Prader-Willi syndrome, and Angelman syndrome) are linked to ART [reviews by Jiang et al. (10), Gosden et al. (37), Maher (38), Odom and Segars (39)], although the absolute risks remain extremely low. In general, an association between ART and altered offspring epigenomes has been reported in several studies (37–41). For example, El Hajj et al. (40) compared children born via ART with naturally conceived children and reported an impact of ICSI on offspring DNA methylation patterns in cord blood, which may contribute toward disease susceptibility in ART children. Nevertheless, the observed risks were low and the study was unable to account for possible confounding effects due to parental age and underlying infertility (see Box 2). Similarly, Gomes et al. (41) reported abnormal DNA methylation patterns in children born after ART, which have also been reported more broadly in animal ART studies (42).

Some studies cited previously [e.g. El Hajj et al (40)] documented small differences in methylomes, and Novakovic et al.'s (43) findings suggest that such limited ART-associated variation in methylation may be generally resolved by adulthood without consequences for long-term health. Nevertheless, this may not be true in all cases. For example, Batcheller et al. (44) reviewed literature on genome-wide epigenetic alterations in phenotypically normal ART offspring and found that ART is associated with widespread epigenetic modifications associated with adverse cardiometabolic outcomes. Furthermore, using a large sample size consisting of 982 ART and 963 non-ART mother-father-newborn trios, Romanowska et al. (45) found evidence for significant altered methylation in girls. Importantly, these methylation alterations were attributable to ART, rather than parental characteristics, as parental DNA methylation and other confounding factors were controlled (45). Furthermore, genes colocalized with the ART-driven epigenetic alterations in the X chromosome reported by Romanowska et al. (45) were linked with key development processes (including neurodevelopment) and intellectual disability, suggesting a causal association between ART and neurodevelopmental outcomes in children [e.g. see Farhi et al. (46)].

ART bypasses female-modulated mechanisms of sperm selection

Almost 4 decades of research on animal reproduction has taught us that females are rarely passive when it comes to the choice of sperm that fertilize their eggs. For example, in polyandrous species (i.e. those in which females mate with more than one male within a single reproductive episode), females can exert physiological control over sperm selection, ensuring that sperm from reproductively compatible, preferred, or genetically superior males are used for fertilization—the so-called cryptic female choice (CFC) hypothesis (47, 48). In particular, there is growing evidence that CFC operates via prefertilization cellular- and molecular-level processes, termed gamete-mediated mate choice (49), and that such mechanisms may operate at the level of individual sperm cells [e.g. Jokiniemi et al. (50)].

Given the extraordinary levels of sperm heterogeneity within a single ejaculate [e.g. Holt and Van Look (51)], it has been argued that CFC may also operate in monandrous species (e.g. humans) by selectively targeting individual sperm cells within an ejaculate (52). Indeed, empirical evidence from the zebrafish *Danio rerio* and the Atlantic salmon *Salmo salar* has revealed that within-ejaculate selection of sperm haplotypes can have fitness benefits for resultant offspring (53–55). For example, in zebrafish, within-ejaculate selection resulted in the lower occurrence of apoptotic cells during early offspring development, higher embryo viability, and fitter offspring (54).

Despite our improved fundamental knowledge of reproduction and CFC, traditional approaches for selecting sperm for ART procedures rely on simple phenotypic selection, for example choosing morphologically normal sperm (56), highly motile sperm (57), or those exhibiting intact DNA (58). The most widely used procedures rely on separation techniques (e.g. swim up, density

centrifugation), which select on motile and morphologically normal spermatozoa (57). More refined separation techniques, for example microfluidic models, have attempted to mimic the physiological conditions of the female genital tract (e.g. pH and temperature) and have the potential to incorporate thermotactic and chemoattractant properties that occur naturally (59). Such devices could potentially be enhanced to mimic the in vivo environment, for example by incorporating cultured oviductal epithelial cells and/or FRFs to better mimic the conditions in the FRT [e.g. see Ferraz et al. (60) and Canha-Gouveia et al. (61) for similar innovation in the context of in vitro embryo culture]. Despite some progress, the most widely used sperm separation techniques bypass critical (and individual-specific) ejaculate-ova interactions (see top of Fig. 1), some of which are known to refine sperm selection during in vivo fertilization (2, 5, 62). Moreover, methods used to select such "normal" sperm focus predominantly on sperm traits thought to improve fertilization prospects rather than the longer-term health prospects of resultant offspring (63–67). Here, we suggest that by bypassing such naturally and sexually selected mechanisms of sperm selection, we risk missing opportunities to select the most appropriate sperm pools for ART.

FRFs and the FRT

In a recent review, Gasparini et al. (68) summarized the key roles that FRFs play in nurturing and extending the fertilization lifespan of sperm and exerting choice among competing sperm (i.e. CFC). For example, irrespective of fertilization mode (internal or external), FRF can play a key role in regulating key physiological processes, including chemotactic responses to egg- or female-derived sperm attractants (69), inducing sperm capacitation and the acrosome reaction (70), mitigating the effects of sperm aging (71), and improving a range of key motility traits that improve fertilization rates (72). FRF also comprise numerous extracellular vesicles, which contain bioactive molecules (e.g. messenger RNA, miRNA, proteins, lipids) that function during intercellular communication and serve important reproductive physiological functions (73–75). There is also evidence that FRF has beneficial effects on the female's eggs, in terms of improving fertility and extending the time available for fertilization (e.g. in zebrafish) (76), although the mechanisms underlying these effects have yet to be established. Collectively, this emerging evidence suggests that the inclusion of FRF during ART procedures may improve overall fertilization outcomes and possibly the health of resultant offspring.

While the previous evidence is suggestive of a *generalized* benefit of incorporating FRF into fertilization protocols (i.e. FRF has positive effects on all sperm within an ejaculate), recent evidence suggests that we should also consider the *individual-specific* benefits associated with the inclusion of FRF and other female-modulated physiological processes during ART. In short, emerging evidence suggests that FRF may facilitate the selection of individual genetically compatible or competent spermatozoa from a single ejaculate. For example, in their review Pérez-Cerezales et al. (77) highlight the role that the mammalian female's oviduct plays in sperm selection and reshaping the epigenetic landscape of the developing embryo, recognizing that even a single ejaculate comprises a heterogeneous mix of different subpopulations of sperm that differ in their epigenomes and DNA quality. Indeed, the role of the FRT in selecting subpopulations of high-quality or genetically "preferable" sperm is increasingly recognized across a range of taxa [see reviews by Holt and Fazeli (3) and Soto-Heras et al. (78)].

Importantly, ART procedures such as ICSI and IVF explicitly bypass such filters, thereby missing opportunities to selectively exclude poor quality or incompatible sperm from the fertilization pool. Intriguingly, recent evidence from *D. rerio* suggests that FRF has the capacity to differentially attract, via chemotaxis, phenotypically and genetically superior sperm populations within a single ejaculate, suggesting that FRF may play a critical selective role in filtering high-quality sperm during in vivo fertilization (79). Similar evidence for within-ejaculate selection of sperm populations by FRF was also reported in mussels (*Mytilus galloprovincialis*), in this case revealing that FRF-selected populations of sperm produce embryos with higher viability than unselected sperm (80).

The potential benefits of incorporating FRF into sperm selection procedures during ART are now being explored, and a number of methods that mimic the selective conditions imposed by the FRT have been considered [e.g. see Baldini et al. (57)]. For example, Homobono et al. (81) reported that supplementation of bovine follicular fluid during IVF resulted in blastocysts that exhibited reduced levels of expression of heat shock protein HSP70 and genes associated with apoptosis, suggesting that adding this fluid to the IVF medium may mitigate stress in bovine developing embryos. Along similar lines, Ahmadkhani et al. (58) assessed the use of microfluidic devices to mimic features of the FRT during sperm selection for ART, concluding that rheotaxis may be an affective mechanism to separate high-quality sperm from the sperm pool. In the light of individual-specific affinities between gametes from compatible mating partners discussed previously (i.e. cellular- and molecular-level CFC), which may extend to within-ejaculate selection of high-quality or compatible sperm (53, 54, 79), we advocate for further development of these systems to include FRF from the affected females undergoing ART. For example, in the future, microfluidic chambers might be modified to incorporate FRF-fueled sperm chemoattraction (82) or cultured oviductal cells [see Ferraz et al. (60)] to ensure that genetically compatible or viable subpopulations of a male's sperm are selected for use in ART. Similarly, the development of sperm-selection protocols designed to reinstate naturally selected filters against sperm carrying deleterious mutations may help reduce the genetic load of ART-conceived offspring (83). These and other innovations in sperm selection technologies [reviewed in Baldini et al. (57)] may enable us to move toward more targeted interventions that improve reproductive outcomes for individuals seeking assisted reproduction.

Gene regulatory processes in the FRT

During ART in humans and other mammals, the ovarian follicular fluid is typically discarded during oocyte retrieval. Yet, the follicular fluid (FF) is a complex fluid that contains numerous factors secreted by surrounding cells, including (as we noted previously) extracellular vesicles that may regulate gene expression. Folliculosomes, for example, comprise numerous miRNAs that are known to intervene in the transcriptional processes of various genes [reviewed by Muñoz et al. (84)]. Indeed, there is emerging evidence from humans that exposure to FF may play an active role in regulating gene expression in mature spermatozoa, possibly via activation of sperm RNA transcription (85). Remarkably, in pigs, early embryos (blastocysts) produced in vitro in the presence of oviductal and uterine fluids exhibited methylation and gene expression patterns that were more similar to in vivo blastocysts, and these resulted in higher quality embryos compared with those arising from in vitro blastocysts that were produced in the absence of reproductive fluid (86). Thus, interventions such as the addition of FRF into the in vitro medium during oocyte maturation and/or at fertilization may

serve to improve the health prospects of ART-derived offspring [e.g. see Canovas et al. (86), da Silveira et al. (87), and Lopera-Vasquez et al. (88)].

Female-modulated immune responses that favor "compatible" sperm

In mammals, the oviduct is the tubular organ that connects the ovaries with the uterus. Although once considered to be a mere passage through which the developing embryo travels on its way to the uterus for implantation, the oviduct is now recognized as playing key roles in affecting gametes, embryos, and ultimately offspring health (78, 89). It has been suggested that the mammalian FRT may serve to select genetically compatible sperm, possibly via immune recognition genes (the so-called molecular passport hypothesis) (3). Suggestive evidence for such immunogenetic gametic compatibility comes from humans, in which studies have shown that the physiological responses of sperm to FF and cervical mucus depend on the level of similarity in human leukocyte antigens, and that human leukocyte antigen–dissimilar male-female combinations are favored (50, 90–92). Similar immunogenetic mechanisms of gametic compatibility have been identified in nonmammalian systems (junglefowl, fish) (93, 94). These findings therefore suggest that mechanisms of gametic compatibility that exploit immunogenetic mechanisms may be evolutionarily conserved across diverse taxa and again suggest that ART approaches that incorporate FRF from the individuals seeking treatment may improve fertilization prospects and subsequent health outcomes for offspring.

Conclusions and future prospects

The key objective of this article is to highlight the general need for researchers and clinicians to take more holistic and biologically inspired approaches when designing and undertaking ART. In short, we ask whether we can we draw on the evolutionary principles of selection when choosing gametes for ART and in designing the environments in which ARTs take place. Although researchers are only beginning to address these questions, we suggest that by applying evolutionary principles to the future development of ART, we may improve outcomes both for human ART and animal production.

In this perspective, we highlight the key roles that FRFs play in both nurturing and selecting optimal sperm during in vivo fertilization and subsequently during embryo culture. Although classic ART methods for sperm selection largely bypass these natural and sexual selection barriers, recent research in the development of microfluidic sperm selection (95, 96) and embryo culture [e.g. (88, 97)] has begun to address these challenges. For example, the experimental supplementation of natural reproductive fluids during swim-up (sperm selection) and embryo culture procedures in pigs and cattle has been shown to result in higher-quality embryos than those produced without the supplementation of FRF (86–88). Translating these findings to humans is clearly a challenge, but as we note, progress has been made in developing protocols that incorporate human reproductive fluids for use in the culture of nonhuman and human embryos (61). We anticipate that similar techniques will be incorporated into sperm selection techniques, for example for use in IVF or intrauterine insemination (57).

While the perinatal outcomes of ART are well established, our review of the literature has highlighted the dearth of studies that consider the longer-term and transgenerational health impacts of ART in human studies. This is not surprising, given that the oldest IVF-conceived human, Louise Brown, is just 46 years of age at the time of writing this review. Consequently, we know nothing about the potential *transgenerational* (i.e. multiple generations) effects of ART in humans, given that the technology has only existed for a few decades. Nevertheless, studies on animal models have provided valuable insights into the potential deleterious transgenerational impacts of ART (98–100), suggesting that future studies on humans should focus on descendants from ART-conceived children. In this regard, when conducting sperm selection protocols, we should strive to prioritize the long-term health outcomes of the offspring (e.g. by "filtering" epigenetically competent sperm) (11) over the traditional focus on fertilization success, birth rates, and similar short-term outcomes (65, 66, 101).

Our review also highlights how reproductive failure can arise through the incompatibility of mating partners, and that selection against such incompatibilities can occur via FRFs found in the FRT. Given the high level of specificity in sperm choice mediated by FRF, can we draw on these same principles when undertaking ART procedures? In a preliminary test of this idea, Lukasiewicz et al. (91) explored the use of nonreproductive human female fluids (serum), which share similar immunoglobulin G and A antibodies as FF. They reported similar physiological responses by sperm to serum and FF, thus potentially pointing to a cost-effective clinical test for gamete-level incompatibility in couples seeking ART (91). Nevertheless, it is important to note that any subsequent intervention that incorporates FRF during fertilization procedures in humans may ultimately reduce the success of ART for those couples (i.e. because FRF may selectively reduce the likelihood of fertilization by reproductively incompatible sperm). Thus, while we anticipate that further advances in diagnostic tests for reproductive incompatibilities will come from studies that combine FRF (or suitable surrogates) with microfluidic systems designed to mimic the FRT [e.g. see Ahmadkhani et al. (58) for a review of current sperm separation methods], the implementation of such procedures during human ART requires careful consideration.

As we note in Box 2, it is important to isolate causal factors associated with ART procedures (e.g. gamete handling, unnatural environments) and the possible role that parental infertility/age can play in transferring epigenetic marks to the offspring. In this respect, research on nonhuman animals will be particularly helpful to tease apart the causality underlying epigenetic effects (102). Notwithstanding research on the conservation biology of species experiencing inbreeding depression (103), nonhuman animals in ART research are not sourced from a subpopulation of individuals with fertility problems, allowing the separation of effects due to ART procedures (e.g. artificial environments) from correlational effects affecting infertile or subfertile subpopulations. Indeed, animal studies have been instrumental in advancing our understanding of the consequences of ART-related procedures (104–111). We therefore advocate for further experimental studies specifically designed to disentangle causation. For example, sperm of healthy and fertile animals can be split (using a within-subject design) between ART and normal conception treatments to look for direct effects of ART independent of any underlying pathology (e.g. associated to infertility). Such designs can include more complex manipulations to further tease apart causal factors, for example environmental conditions established by ART or the role of female modulating factors (e.g. through the presence or absence of FRFs).

Finally, the potential advantages of incorporating evolutionary principles when designing ART procedures will extend beyond the clinical and human reproduction sectors. We suggest that IVF

procedures carried out during animal production could be guided by a more nuanced understanding of evolutionary principles, including epigenetic inheritance, individual-specific patterns of mate choice, CFC via sperm-FRT interactions, and so on. For example, in commercially important externally fertilizing species, we know that FRF can have individual-specific chemical profiles that selectively upregulate and/or attract sperm from genetically compatible males (112, 113). Thus, simply incorporating gamete choice and/or competition protocols into IVF processes (e.g. in aquaculture) might boost the resilience and productivity of commercial breeding programs (114).

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Author Contributions

Conceptualization, writing and editing: J.P.E. and F.G.-G.

Data Availability

There are no data underlying this work.

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