Reproductive Medicine has been at the forefront of the biotechnology revolution. Over the last three decades, a very significant proportion of abstracts at our major meetings and papers in our journals have employed molecular techniques to elucidate basic gene expression and cellular metabolic pathways in the study of reproductive processes. The resulting insights have yielded new understanding of the basic mechanisms of reproductive diseases and innovative therapeutic modalities. Specific examples will be used to illustrate this remarkable progress.

In 1988, Hollands reported the rescue of lethally irradiated rats by infusion of haemopoietic stem cells from mouse blastocysts grown in vitro (1). This remarkable study was published decades before stem cells (now induced pluripotent cells from the recipient to avoid rejection) became a clinical tool for repopulating the bone marrows of cancer patients following lethal doses of chemotherapy and or radiation.

In 1992, Lou Ignarro, who later shared a Nobel Prize for the discovery of nitric oxide (NO), showed using human penile tissue that the effect of neural NO release was enhanced by an inhibitor of cyclic GMP degradation (2), leading to the development of highly effective treatments for erectile dysfunction (ED) (3). Further elucidation of the factors influencing NO production and degradation has led to important insights into lifestyle and nutritional factors influencing erectile health (4, 5).

In the mid-1990’s, as the result of work carried out by the research group under Antonio Pellicer in Valencia, Spain and others, the etiology of increased vascular permeability and consequent extreme fluid shifts characteristic of ovarian hyperstimulation syndrome (OHSS) were shown to be due to increased expression of vascular endothelial growth factor (VEGF) and VEGF receptor2 (VEGFR-2) (6). The incidence of OHSS was reduced by 50% by identifying and clinically utilizing the role of dopamine agonists to inhibit phosphorylation of VEGFR-2. That discovery, together with understanding and thereby avoidance of clinical factors stimulating VEGF, has now made morbid and potentially fatal OHSS a rare occurrence.

Again in the 1990’s, David Gardner, along with others, defined the reproductive tract nutrients available during in vivo embryo development and the specific stage-specific requirements of embryos developing to the blastocyst stage. Those insights and clinical experience enabled the design of media specifically for embryos over the first 72 hours and the following 24 to 84 hours of culture (7), improving IVF efficiency and making accurate chromosome analysis a reality. Study of blastocyst metabolism that predicts a successful pregnancy has been progressing rapidly (8).

In the late 1990’s techniques became available to assess DNA fragmentation of sperm, first by flow cytometry and later by techniques such as TUNEL and COMET. DNA fragmentation increases with age (9) and may contribute to the low success rates in women over age 40, although the effect of male age was observed to be less with younger female age (10). The effect on IVF outcome also appears to be less for women responding normally to ovarian stimulation compared to low responders (11). These observations have suggested that cytoplasm of the better quality oocyte may be able to correct DNA fragmentation. The observation that DNA fragmentation is correlated with semen oxidative stress (12) has validated successful treatment with antioxidants (13). Because DNA fragmentation increases during transit through the male collecting system, testicular extraction of sperm has been used successfully if frequent ejaculation and antioxidants are not sufficient (14). Decreased coital frequency with age, in part due to decreased erectile function, contributes to infertility in older couples (15).

In the late 1990’s the importance of omega-3 fatty acids in the function of sperm membranes was being elucidated and correlated with indices of sperm quality and male infertility (16). Safarinejad reported a randomized trial showing highly significant benefits of omega-3 supplementation on sperm density, motility, and strict morphology (16). Semen concentrations of omega-3’s correlated with those improvements and also with semen endogenous antioxidants. Supplementation with omega-
Implantation is disrupted (23), leading to the suggestion that ovarian stimulation, expression of genes involved in production. Endometrial gene arrays have been carried to be fully explored (22).

Elagolix, and newer even more potent molecules, have yet ple, the oral gonadotropin-releasing hormone antagonist, of this and other non-invasive oral treatments, for exam- relieved pain and improved quality of life (21). The place progesterone receptor modulator, achieved amenorrhea 12 week courses of 5 and 10 mg of ulipristal, a selective expression of estrogen on myoma growth. Repeated torics and comprehensive chromosome screening (CCS) of all chromosomes in high quality, experienced laborato ries appear to be highly successful. Effective single em- broyo transfer of a euploid embryo then avoids the very sig- nificant morbidity and perinatal mortality accompanying twin births while also reducing miscarriage (18). Newer CCS platforms allowing greater resolution (particularly next generation sequencing, NGS) have highlighted the importance of a high quality laboratory in producing blastocysts with fewer mitotic errors that result in embryo mosaicism (19). By avoiding the stress of twins, futile transfers, and preventable miscarriages, fewer couples may further compromise their success by dropping out of treatment (20). It is important to point out that data generated from registries that include a broad range of IVF laboratory quality and data collected before full optimization of the em- broyo culture environment must not be used to cast doubt on the importance of this breakthrough in IVF treatment (19).

Uterine leiomyomas have long been a major reproduc- tive obstacle and a source for gynecologic morbidity and need for major surgery. Until recently, non-invasive tech- niques have had limited success, with ovarian estrogen suppression having transient benefits and bothersome side effects, and uterine artery embolization not being applic- able to women planning to conceive. Molecular tech- niques have shown that progesterone (P) is a key stimula- tor of myomas and activation of myoma P receptors may express the effect of estrogen on myoma growth. Repeated 12 week courses of 5 and 10 mg of ulipristal, a selective progesterone receptor modulator, achieved amenorrhea in 62% - 73%, shrinkage of myoma volume by 54% - 58%, and relieved pain and improved quality of life (21). The place of this and other non-invasive oral treatments, for exam- ple, the oral gonadotropin-releasing hormone antagonist, Elagolix, and newer even more potent molecules, have yet to be fully explored (22).

Implantation has always been the “black box” of re- production. Endometrial gene arrays have been carried out during natural and ovarian stimulation cycles. During ovarian stimulation, expression of genes involved in implantation is disrupted (23), leading to the suggestion that embryo transfer might be more successful during natural cycles or with hormone replacement to mimic the normal menstrual cycle. Results from a randomized study showed an increase of ongoing pregnancy from 51% to 78% (P 0.007) (24). The development of cryopreservation by vitrification, which has minimal effects on embryo capac- ity, has spurred a “sea change” toward deferred transfer of embryos, also promoted due to some evidence pointing to better perinatal outcomes, clear evidence for a reduced rate of ectopic pregnancy, and the ability to send out biop- sies from day 5 to 7 blastocysts to reference laboratories. More recently, gene array has been used to identify indi- vidual women whose endometrial “window of implantation” is shifted, resulting in capable embryos failing to im- plant. Thus, a personalized approach to the timing of em- broyo transfer has recently been reported in a multicenter randomized trial to improve outcome in women with recur- rent implantation failure (25).

New approaches are emerging for women who have lost their ovarian function due to chemotherapy or pre- mature ovarian failure. By cryopreserving ovarian tissue prior to cancer treatment, replacement of that tissue at a later time into or adjacent to the ovary has now resulted in natural and IVF pregnancies as well as hormonal function and menses. Globally over 70 births have been achieved (26). For premature ovarian failure various approaches are being explored based on knowledge of the molecular pathways controlling follicle growth. U.S. and Japanese re- searchers have activated follicles in ovarian cortex biopsies by disrupting Hippo signaling by fragmenting the tissue, followed by enhanced Akt signaling and autografting. IVF then resulted in a normal birth (27). Others have been ex- perimenting with infusion of various stem cells and their derivatives from bone marrow or umbilical cord tissues into the ovaries. Recently chemotherapy treated mice had rescue of ovarian function and fertility by intra-ovarian in- jection of umbilical cord mesenchymal stem cells (28).

The aging oocyte has reduced capacity and is prone to chromosomal segregation errors due in part to im- paired mitochondrial energy production. In a very de- tailed study in aging mice, co-enzyme Q-10, a key enzyme in mitochondrial energy production, was shown to largely reverse those aging changes, resulting in improved ovar- ian reserve and spindle formation and increased litter sizes (29). A limited study in the human showed improved outcomes that were not statistically significant but the numbers were small and the CoQ-10 was taken for only 2 months (30). Poor mitochondrial function is also asso- ciated with overproduction of mitochondrial DNA copy number, which is now used clinically to identify embryos that are unlikely to implant (31, 32).

The ultimate biotechnology goal in reproduction is the
generation of oocytes and sperm from pluripotent stem cells. Oocytes have been produced from pluripotent germ cell-like cells developed from the animal’s own induced pluripotent cells resulting in births of normal, fertile mice, although requiring some assistance during development by ovarian supporting cells in vivo (33). Sperm have been produced from mouse embryonic stem cells using in vitro co-culture with neonatal testicular cells, resulting in viable, fertile offspring (34). Also encouraging is the identification and maturation of pluripotent germ cell-like cells from testicles lacking mature sperm. Extensive efforts by groups around the world were recently detailed in a systematic review (35). Clinical use of stem cell-derived gametes will require detailed research using animal models because of the genetic and epigenetic/developmental risks involved in assuring that such cells are fully normal.

In summary, a dozen examples have been given of the remarkable progress in understanding and treating disorders of reproduction. Researchers in reproductive medicine have earned their place as pioneers in the application of biotechnology to the health sciences.

References


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