JPET Fast Forward. Published on March 15, 2005 as DOI: 10.1124/jpet.104.080051

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The Evolution of In Vitro Fertilization: Integration of Pharmacology, Technology, and Clinical Care

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Running Title: Evolution of in vitro Fertilization

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Text Statistics:

Number of Text Pages:	18	Tables:	1
Words in Abstract:	196	Figures:	3
Words in Article:	4773	References:	62

Abbreviations: COH, controlled ovarian hyperstimulation; IVF, in vitro fertilization; OHSS, ovarian hyperstimulation syndrome; FSH, follicle stimulating hormone; LH, luteinizing hormone; hCG, human chorionic gonadotropin; hMG, human menopausal gonadotropin; GnRH, gonadotropin releasing hormone

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This article has not been copyedited and formatted. The final version may differ from this version.

ABSTRACT

For the couple having trouble achieving pregnancy, the options and opportunities for assistance have never been brighter. Options such as controlled ovarian hyperstimulation, in vitro fertilization and intracytoplasmic sperm injection have been developed over the past five decades and provide hope for couples that previously would have been considered infertile. In vitro fertilization and intracytoplasmic sperm injection represent a coalescence of advances in physiology, endocrinology, pharmacology, technology, and clinical care. In vitro fertilization has assisted well over one million couples in their efforts to start or build a family, and the demand for such services continues to increase. The purpose of this manuscript is to review the pharmacological advances that made controlled ovarian hyperstimulation, and therefore in vitro fertilization and intracytoplasmic sperm injection, possible. We will discuss the early stages of gonadotropin use to stimulate ovarian production of multiple mature eggs, the advances in recombinant technology that allowed purified hormone for therapy, and the use of other hormones to regulate the menstrual cycle such that the likelihood of successful oocyte retrieval and embryo implantation is optimized. Finally, we will review current areas that require particular attention if we are to provide more opportunity for infertile couples.

INTRODUCTION

Controlled ovarian hyperstimulation (COH), in-vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) have become the standard of care for many couples with infertility. The combination of pharmacologic and surgical manipulation of the menstrual cycle is the key to improvement in pregnancy rates. The history and evolution of COH, IVF and ICSI has been rapidly developing and continues to change at an ever increasing pace (Fig. 1). As we learn more about the molecular basis of cellular communication and signaling, pharmacologic treatments with greater efficacy can be developed. Improvements in embryo culture techniques and embryo cryopreservation will similarly enhance outcomes. The purpose of this review is to summarize the history and development of the pharmacology that has made COH and IVF successful.

In a typical menstrual cycle there is formation of a single dominant follicle, from which ovulation of a single oocyte occurs each month. For the fertile couple, this menstrual cycle has a 20% chance of resulting in a pregnancy. However, for infertile couples the chance of pregnancy with one oocyte can be well under 5% per cycle. Over the past 50 years, pharmacologic agents have been developed to increase the likelihood of pregnancy by increasing the number of eggs released and available for fertilization.

To clarify the obstacles to fertility that have been overcome as well as the challenges that remain, we will review the physiology of the menstrual cycle, major historical developments, the therapeutic evolution and current use of medications used during controlled ovarian hyperstimulation and in-vitro fertilization. This review, by design, will be a brief overview. Those readers interested in specific detail on topics such as cryobiology (Fuller and Paynter, 2004), intracytoplasmic sperm injection (Lewis and Klonoff-Cohen, 2005), embryo culture media (Summers and Biggers, 2003), preimplantation genetic diagnosis (Sermon et al., 2004), diminished ovarian reserve (Derman and Seifer, 2003), ovarian hyperstimulation syndrome (Avecillas et al., 2004), male-factor infertility (Nicopoullos et al., 2004), or nuclear transfer (Burmeister et al., 2001) are referred to the listed reviews for further detail.

Menstrual cycle physiology

The menstrual cycle supports the growth and maturation of oocyte containing follicles, and can be split into two phases: the follicular phase and the luteal phase. The follicular phase usually is 14 days and is characterized by hormonal interaction to ensure development of a single follicle for ovulation (Palter and Olive, 1996). The luteal phase is also 14 days and begins with ovulation, or release of the oocyte from the follicle, and ends with menstrual flow or pregnancy.

The oocyte is contained in a fluid filled space called a follicle that contains two major cell types, each responsible for the production of different hormones. The granulosa cells are responsible for the conversion of androgen to estrogen and are most responsive to the follicle stimulating hormone (FSH) while the theca cells produce androgens and are most responsive to luteinizing hormone (LH). The interplay between FSH, LH, androgens and estrogens is critical for ovulatory function and menstrual cycle regulation.

The sequence of events that culminates in ovulation involve a series of well regulated critical steps. In order for ovulation to occur, a single follicle must be recruited and its growth promoted. In order to be selected, a follicle has to first undergo conversion from a primordial follicle to a pre-ovulatory follicle. Girls are born with a pre-determined number of primordial follicles, usually millions, which have the capability of becoming pre-ovulatory follicles beginning at puberty. These immature primordial follicles need ten weeks to develop into more mature pre-ovulatory antral follicles (Hillier et al., 1985). Primordial follicles are surrounded by only a single layer of granulosa cells. Increases in FSH stimulate increased numbers of granulosa cells and thereby increased production of estrogen. With granulosa cell hyperplasia, the primordial follicles become antral follicles that have many layers of granulosa cells and are capable of producing high levels of estrogen. The selection of a single dominant follicle from the pool of available follicles is determined by FSH responsiveness. The follicle that is the most sensitive to FSH will become dominant by up-regulating FSH receptors on granulosa cells and increasing granulosa cell numbers. A higher concentration of granulosa cells enables production of more estrogen, and the

increased estrogen has a negative feedback effect on FSH production by the pituitary, which decreases FSH secretion (Fig. 2). Because the dominant follicle has a higher number of FSH receptors and peak FSH sensitivity it will be able to respond despite lowering FSH levels. Other developing follicles cannot survive with the reduced FSH secretion and undergo apoptosis and follicular atresia. Receptors for LH appear on the dominant follicle as it approaches ovulation, and a pituitary-mediated LH surge advances oocyte meiosis and ovulation. The act of ovulation signals the beginning of the luteal phase. The residual ovarian follicle is transformed into the corpus luteum which produces progesterone. Progesterone thickens the endometrial lining and helps an embryo implant and grow. If fertilization ensues, placental production of human chorionic gonadotropin (hCG) supports corpus luteum progesterone production until the placenta takes over progesterone production at 8-9 weeks. If the ovum is not fertilized, hCG is not produced, the corpus luteum regresses, and progesterone production is halted. The absence of progesterone leads to withdrawal of support of the endometrial lining and menstruation begins. Each of these processes is tightly controlled.

Pharmacotherapy takes advantage of the distinct roles of FSH and LH in all phases of the menstrual cycle including follicular recruitment, oocyte maturation, ovulation and luteal support. Clomiphene citrate is the most commonly used medication to alter follicular recruitment and was first synthesized in 1956 and approved for clinical use in the USA in 1967. Clomiphene acts on the hypothalamus; its anti-estrogenic nature promotes a subsequent increase in release of pituitary gonadotropin secretion and recruitment of more than one dominant follicle (Dickey and Holtkamp, 1996). Clomiphene does not directly stimulate ovulation, but rather, it modifies and amplifies the sequence of naturally occurring events. Today, Clomiphene is used mainly as a first line treatment for infertility. Gonadotropin treatment using purified or recombinant FSH and LH are used in superovulation for COH and IVF regimens. Luteal support can be enhanced with the use of Clomiphene, hCG or progesterone.

Pharmacologic control of the menstrual cycle

Human Chorionic Gonadotropin

A primitive understanding of pituitary control of the menstrual cycle evolved early in the 20th century. It was first demonstrated that partial ablation of the pituitary resulted in atrophy of the genital organs in adult dogs as well as maintenance of sexual infantilism in puppies (Crowe et al., 1910). A hypothesis for function of the pituitary-gonad axis was subsequently developed with speculation that the pituitary secretes two hormones that stimulate the ovaries. The secretion of these hormones was also noted in the urine of menopausal women and the concept of "negative feedback" in the hypothalamic-pituitary-gonad axis was pioneered (Gemzell, 1965). Discoveries of other reproductive hormones were rapidly emerging and scientists were working to elucidate these endocrine pathways. Human chorionic gonadotropin (hCG), a hormone central to pregnancy maintenance, was next discovered and was initially thought to originate from the anterior pituitary. Subsequently, it was demonstrated through the use of tissue culture techniques that hCG is produced by the placenta rather than the pituitary (Seegar-Jones et al., 1943). hCG and LH are both dimeric glycoproteins composed of two non-covalently linked subunits, alpha (α) and beta (β). Since the β subunits between LH and hCG are similar, these glycoproteins have identical mechanisms of action via interaction with the same receptor. Physiologically, the similarity in mechanism of action allows hCG produced by the placenta to stimulate the LH receptors present on cells within the corpus luteum to produce progesterone, which inhibits uterine contractility and promotes immune quiescence. The hCG for commercial use was purified from the urine of pregnant women beginning in the 1940's (Gurin et al., 1940). While LH has a half-life of 30 minutes, hCG has a half-life of greater than 24 hours (Strott et al., 1969). Due to its longer half life and ability to promote ovulation in a manner similar to LH, hCG was the first agent used for ovulation induction in humans (Hamblen, 1939).

Pituitary Extracts of Gonadotropins

In addition to the use of hCG for ovarian stimulation, pituitary extracts of FSH and LH were developed for use in ovulation induction. In 1956, Maddock and colleagues described enlarged cystic ovaries in response to hog pituitary FSH administration (Maddock et al., 1956). Two years later Gemzell and

colleagues first described the use of human pituitary extracts plus hCG in amenorrheic women (Gemzell et al., 1958). Various parameters were employed to provide proof of end-organ effects of pituitary hormones. They demonstrated polycystic appearance of the ovaries, changes in endometrium histology, and measurements of urinary excretion of estrogen and progesterone by-products. In addition, they were able to successfully induce resumption of menstruation in this amenorrheic population, further demonstrating the ability to induce ovulation. (Gemzell et al., 1958). Human derived pituitary extracts were superior to animal products because of the lack of antibody formation and subsequent time-related decrease in efficacy (Jungck and Brown, 1952), but were exceptionally difficult to procure. The use of human pituitary extract continued for three decades until cases of iatrogenic fatal Creutzfeld-Jacob prion disease arose and were linked to the use of unprocessed pituitary extract (Cochius et al., 1990). Fortunately, alternative methods of safely isolating FSH and LH for use in ovulation induction were already under development, as described below.

Urinary Extracts of Gonadotropins

Menopausal ovaries exhibit a marked decline in estrogen production. As a result, pituitary FSH and LH secretion is no longer inhibited and high serum levels and increased urinary excretion ensues. Donini and colleagues described techniques to extract these hormones from the urine of menopausal women for biologic use in women of reproductive age (Donini et al., 1964). The first preparation of human menopausal gonadotropins (hMG) for clinical use was released in 1950 (Table 1). In 1953, hMG was used in hypophysectomized rats and in the 1960's its use was described in humans.

While hMG revolutionized fertility treatment, adverse effects such as formation of lutein cysts and multiple pregnancies were reported (Pasetto and Montanino, 1967). The exact composition of hMG remained an area of debate; were FSH and LH one compound, or could they be separated? Donini and colleagues postulated that separation of FSH and LH would provide improved hormonal control and therefore improve clinical outcomes(Donini et al., 1966). In a series of elegant experiments, he described the purification and separation of FSH and LH from HMG by taking advantage of the

structural similarities of LH and hCG and using hCG antibodies to bind LH. Donini thereby pioneered the movement towards a more highly purified FSH.

Clarification of the unique roles of FSH and LH provided further insight into reproductive regulation. Several groups evaluated the function of highly purified FSH alone compared with highly purified FSH supplemented with LH (in the form of hMG) (Gemzell et al., 1958; Eshkol and Lunenfeld, 1967). They found that while the mice exposed to hMG had enlarged ovaries, enlarged follicles and increases in uterine size, mice who received FSH alone also had stimulated ovaries, but had no changes in uterine size. These results demonstrated that FSH in the absence of LH is incapable of stimulating uterine growth. The overall consensus was that both FSH and LH were both critical; however, each has different biologic activity. Research has continued over the past five decades and continues today to further elucidate these different roles.

Purified Gonadotropins

The next step after achieving separation of FSH and LH was to focus on FSH purification in order to ensure consistency and reduce batch to batch variability. Early purification techniques were first created using enzymes to digest LH and were not completely successful. Subsequent attempts at purification were more effective utilizing gas column chromatography and gel electrophoresis (Donini et al., 1966a; Donini et al., 1966b). Purification of hormones allowed for more tailored stimulation protocols, increased ease of administration, and subcutaneous injection due to lower protein concentration. Major breakthroughs in purification arose with the use of recombinant DNA technology. The gene encoding the FSH molecule was cloned and placed in an expression vector that allowed the production of large quantities of purified hormone. Unlike other attempts to create a purified product, recombinant FSH has absolutely no LH. Recombinant FSH was developed and approved for clinical use in the mid 1990's and led to improved control of gonadotropin exposure (Daya, 2002). Recombinant LH (rLH) has been used in Europe since 2000 and was FDA approved for use in combination with FSH for the treatment of

hypothalamic hypogonadism in the United States in 2004. Further studies are needed to evaluate the role, if any, of rLH during ovarian stimulation protocols for IVF.

Use of Gonadotropins and Pregnancy

Human menopausal gonadotropins were first used for ovarian stimulation protocols before the development of in-vitro fertilization technology. Pregnancies were achieved, but initial optimism was tempered by the sudden increase in high order multiple gestations (Neuwirth et al., 1965). In vitro fertilization gave the couple control of the number of embryos reaching the uterus, thereby decreasing the risk of multiple embryo implantation. In 1976, the first pregnancy with ovarian stimulation and in-vitro fertilization occurred, resulting in a tubal ectopic implantation (Steptoe and Edwards, 1976). Two years later, an in-vitro fertilization cycle was successfully performed and led to the birth of Louise Brown (Edwards et al., 1980). Since that time, IVF has fulfilled the promise of pregnancy attainment for literally millions of otherwise infertile couples. The steps required for success involve interplay of physiology, pharmacology, and technology.

Down regulation of GnRH

Physicians and scientists became increasingly aware of challenges with ovarian stimulation cycles and sought to refine these protocols. Much progress had already been made in the area of follicular recruitment and stimulation, yet there remained biologic processes which were interfering with the success of in-vitro fertilization cycles. A classic example is the LH surge: the release of LH from the pituitary that results in ovulation. In order for successful oocyte collection during in-vitro fertilization, the physician must surgically retrieve the oocytes from the ovary *before* ovulation occurs. In early IVF cycles, premature ovulation occurred 50% of the time rendering oocyte collection impossible. Pharmacologic interventions that block the LH surge allowed for better cycle control, decreased cancellation rates and increased success.

Gonadotropin-Releasing Hormone Agonists

The LH surge is driven by estrogen's positive feedback on the hypothalamus and pituitary. Follicular estrogen secretion increases the pulsatile release of hypothalamic gonadotropin releasing hormone (GnRH), which stimulates pituitary LH secretion. This process starts slowly and escalates, culminating in a surge of LH followed by ovulation 36 hours after this surge. Inhibition of the LH surge is possible by interfering with GnRH signaling. Work in primates by Belchetz and colleagues demonstrated that a constant infusion of GnRH suppressed the secretion of FSH and LH (Belchetz et al., 1978). Administration of native GnRH mimics hypothalamic tonic secretion preventing the LH surge by down-regulating GnRH receptor signaling. Unfortunately, endogenous GnRH has an eight minute half-life, making tonic levels difficult to maintain. This problem was overcome by the production of GnRH analogues that resisted degradation. The amino acid sequence of GnRH was characterized in 1971 by Schally with crucial functions associated with precise sequence (Schally et al., 1971). The 1, 2, 3, 6, and 10 positions were found to be vital for GnRH function with positions 2 and 3 mediating gonadotropin release and positions 1 and 6 responsible for three dimensional structure (Coccia et al., 2004). GnRH analogues were synthesized by substituting other amino acid bases or complex molecules at the 6 (Gly) and/or the 10 (Gly) positions (Fig. 3). This enables longer biologic activity via resistance to enzyme degradation by endopeptidases and greater GnRH receptor affinity (Pimstone et al., 1977). The newly developed agonists have effects that last weeks to months (Periti et al., 2002).

Pituitary down-regulation and decreased FSH and LH secretion is not immediate upon administration of a GnRH agonist (GnRH-a). These agents first bind and activate the initially functional GnRH receptors with a resultant increase in FSH and LH secretion. This is often referred to as the "flare effect" because of its ability to cause a brief period of ovarian stimulation. With continuous binding, however, the GnRH receptors are internalized and pituitary gonadotrophs become insensitive to further stimulation.

Once GnRH agonists were created, their use quickly became widespread. Meldrum and colleagues first published the use of GnRH-a to create a "medical oophorectomy" and Yen described the diverse use of GnRH-a in humans, including the use in males (Meldrum et al., 1982; Yen, 1983). Porter and

colleagues first used GnRH-a for ovulation suppression in in-vitro fertilization cycles (Porter et al., 1984). Wildt and colleagues similarly used GnRH-a in patients who had previously failed IVF due to premature LH surges, resulting in successful completion of all IVF cycles (Wildt et al., 1986). Gonadotropin releasing hormone agonists greatly improved success rates as well as convenience in scheduling IVF cycles. With the advent of these medications, groups of patients were synchronized and underwent ovarian stimulation together allowing for pre-determination of cycle scheduling and prediction of time to oocyte retrieval.

As physicians became more comfortable with the use of GnRH agonists, they developed protocols whereby agonists were started in either the follicular or luteal phase of the menstrual cycle. Follicular phase protocols take advantage of the "flare effect" while luteal protocols have longer term down-regulation with more pronounced diminution of ovarian activity. The decision regarding which protocol to use is largely determined by a patient's age and ovarian reserve status. It is well documented that fecundity and ovarian reserve decline with age (Tietze, 1957). Ovarian reserve refers to the pool of primordial follicles remaining within the ovary and although it is largely dependent on age, it can be affected by surgery, chemotherapy or other unknown factors. Assessment of ovarian reserve prior to IVF is crucial both for optimizing stimulation protocols and prediction of IVF success (Akande et al., 2002). Day 3 FSH levels as well as clomiphene citrate challenge tests are used today as ovarian reserve predictors (Toner et al., 1991; Hofman et al., 1996).

Patients with diminished ovarian reserve are often placed on follicular protocols to use the initial flare to boost endogenous LH and FSH production. Down regulation occurs after this brief stimulatory period and the LH surge is effectively prevented. Patients with normal ovarian reserve and especially those with polycystic ovarian syndrome who could be at risk for hyperstimulation syndrome are placed on luteal protocols.

GnRH antagonists

Women undergoing luteal protocols require daily GnRH injections for 20-30 days for down regulation and LH surge prevention. Another option would be to allow endogenous gonadotropins to enhance stimulation in the first part of the follicular phase until there is a need to inhibit the LH surge. The GnRH antagonists (GnRH-ant) fit this role well and were first described and used for LH surge inhibition during unstimulated, or "natural" IVF cycles (Meldrum et al., 1994). The GnRH antagonists gained popularity because they were found to have equal efficacy in LH surge prevention with the advantages of shorter duration of stimulation, fewer injections, smaller doses of gonadotropins, and improved patient tolerability with decreased side effects such as hot flashes (Fluker et al., 2001).

While GnRH-a usually only have a single amino acid substitution, GnRH-ant have substantially different structures with multiple amino acid substitutions (Fig. 3). The first generation of GnRH-ant had the disadvantage of anaphylaxis secondary to histamine release from the combination of the hydrophobic N-terminus and basic/hydrophilic C-terminus (Ljungqvist et al., 1987; Flouret et al., 1992). Third generation GnRH-ant (cetrorelix, ganirelix) have amino acid substitutions in these and other positions and do not induce histamine release (Table 1). The antagonists competitively inhibit GnRH release via binding with the GnRH receptor and inhibiting signal transduction and gonadotropin secretion. Unlike native GnRH or the GnRH agonists, the antagonists do not have the ability to activate the receptor, causing an immediate decline in LH and FSH levels which is apparent within several hours of administration.

Most recently, the use of GnRH-ant in poor responders has been studied. There is controversy in the literature as to whether or not antagonist use leads to lower cancellation rates and improved pregnancy outcomes. Several studies have shown a clear benefit while others have not (Fasouliotis et al., 2003; Loutradis et al., 2004) Randomized controlled trials comparing pregnancy outcomes with GnRH agonists and antagonists in poor responders are needed.

Oral Contraceptive Pills

Although initially invented to prevent conception, oral contraceptive pills (OCPs) have become an integral part of timing and coordination of IVF cycles. They were first developed in the 1950s and used clinically beginning in the 1960s. They are a mixture of a synthetic estrogen (desogestel, ethinyl estradiol or mestranol) and one of several C-19 steroids with progestational activity (Baird and Glasier, 1993). The main mechanism of action is through inhibition of GnRH release from the hypothalamus and suppression of pituitary release of LH and FSH (Mishell et al., 1972). As the use of oral contraceptives increased, non-contraceptive health benefits became more apparent and they were being prescribed for wider indications such as menorrhagia, reduction of anemia, dysmenorrhea, and prevention of ovarian cyst formation. Chronic inhibition of ovulation was also discovered to have protective benefit against ovarian and endometrial cancer (Burkman et al., 2004). It is precisely this mechanism, inhibition of the LH surge, which also made OCPs an integral part of the pharmacology routinely used in IVF cycles.

Initial attempts with in vitro fertilization involved oocyte retrieval during a normal menstrual period. This technique was limited by the frequent difficulty in timing the menstrual period precisely, the small numbers of oocytes retrieved, and the need for an IVF team to be available at all times. The use of gonadotropins improved the yield of oocytes, but the risk of premature LH surge resulted in a significant failure rate. Addition of GnRH agonists and antagonists decreased the risk of premature LH surge, but could not be used to coordinate cycles due to cost, frequent injections, and long-term side-effects. As a result, a relatively inexpensive, well tolerated means of controlling the menstrual cycle was needed and oral contraceptive pills provided such a therapy. Frydman and colleagues were one of the first groups to use OCPs for this indication and they reported a preset schedule of down-regulation with OCPs followed by fixed-stimulation and retrieval date (Frydman et al., 1986). Other investigators described similar regimens with marked improvements in scheduling and without differences in pregnancy rates (Patton et

al., 1988). Oral contraceptive pills are routinely used by most programs to overcome the need to rely on a woman's natural cycle. They are begun 1-2 months prior to the scheduled start date of IVF. Oral contraceptive pills are used to coordinate timing of onset of IVF cycles and in combination with GnRH agonists/antagonists remove most potential difficulties with regard to scheduling, helping both patients and staff.

Progesterone supplementation

Although pituitary down regulation and suppression of the LH surge is beneficial for preventing premature ovulation, the disadvantage is a resultant dysfunctional corpus luteum. As discussed earlier, the corpus luteum is the transformed ruptured follicle responsible for progesterone production and support of the pregnancy until the placenta assumes this role. Disruption of the follicle by oocyte aspiration can result in deficient luteal phase support. This iatrogenic luteal phase defect can decrease implantation and pregnancy rates (Macklon and Fauser, 2000). In an attempt to compensate for this detrimental effect, practitioners have supplemented the luteal phase using a variety of hormonal agents. Meta-analysis by Soliman and colleagues in 1994 evaluated more than 30 randomized clinical trials addressing the need for luteal phase supplementation in IVF cycles, and concluded that the use of intramuscular hCG or progesterone led to significantly higher pregnancy rates than placebo (Soliman et al., 1994).

Meta-analysis by Pritts and Atwood in 1992 limited the evaluation to trials using GnRH agonist down-regulation, and found that use of hCG and progesterone resulted in similar pregnancy rates (Pritts and Atwood, 2002). However, they concluded that intramuscular progesterone was the preferred agent for luteal phase supplementation over hCG, as it does not have a direct ovarian stimulatory effect which can cause an increased incidence of ovarian hyperstimulation syndrome. Other studies have similarly shown hCG to be a promoter of OHSS and therefore favor the use of progesterone (Araujo et al., 1994). Pritts

and Atwood stopped short of addressing the question of how long luteal support should be continued. Surgical removal of the corpus luteum has been shown to cause miscarriage until to the 7th week of pregnancy; however, it has not been convincingly demonstrated that luteal support beyond 2 weeks after embryo retrieval is indicated (Penzias, 2002).

The most controversial, and perhaps most clinically relevant, debate over luteal support with progesterone is the route of administration. Most practitioners continue to use intramuscular progesterone in oil, given that it has been widely tested, and small doses (25 – 50 mg) can maintain high and sustained "luteal range" serum concentrations. However, patients dislike the daily intramuscular injection and injection-related reactions. Oral progesterone has been discounted as a viable method of administration. It undergoes nearly complete degradation during hepatic first-pass metabolism, (Pritts and Atwood, 2002) and all randomized trials comparing oral progesterone to parenteral routes of administration have shown significantly lower pregnancy rates.

Vaginal progesterone, however, provides an attractive alternative to the poorly tolerated intramuscular form. It is easily administered, is the preferable route of administration to both physicians and patients, and it avoids hepatic first-pass metabolism. While vaginal progesterone leads to lower serum concentrations, a higher concentration of progesterone is achieved at the uterus – a phenomenon which has been labeled the "uterine first pass effect" (Ludwig and Diedrich, 2001).

Vaginal progesterone is available in several different formulations. Micronized tablets (Prometrium), or capsules (Utrogest, Utrogestan) have been studied in doses of 200 – 600 mg daily (Table 1). More recently, a lipophilic gel preparation (Crinone 8%), has been studied at a 90 mg daily dosage. All

formulations have been shown to adequately synchronize the endometrium in the luteal phase, but result in significantly lower pregnancy rates when compared with intramuscular progesterone. Given this data, most clinicians still rely upon intramuscular progesterone for luteal phase support and only use vaginal formulations in women who are unable to tolerate the intramuscular route. However, given the lesser tolerability and side effects of the intramuscular formulation, debate over the use of vaginal progesterone will likely continue.

Future Directions

While advances in pharmacology created the opportunity for improving fertility rates in couples having trouble conceiving, there remain significant limitations that could potentially be addressed by improved understanding of the factors affecting oocyte and endometrial quality and function. Even in the optimal couple, the likelihood of clinical pregnancy with repeated IVF cycles is 80%. The remaining 20% likely have currently undetected defects in the endometrial lining that prevent implantation of otherwise viable embryos. Currently, our understanding of embryo implantation is rudimentary, but several proteins may serve as targets for pharmacological agents that result in improved embryo tethering and invasion. Embryo implantation begins via blastocyst apposition to the uterine wall followed by trophoblast migration and invasion of the underlying uterine tissue (Red-Horse et al., 2004). This enables a connection to be established between fetal and maternal circulation and creates placental blood supply. Recent studies have identified L-selectin, which mediates tethering of leukocytes on blood vessels, as having a critical role in blastocyst implantation (Genbacev et al., 2003).

Many women with infertility problems are so burdened because they choose to delay pregnancy. Biologically, optimal fertility occurs in the late teens and twenties (Tietze, 1957). Many women in this time-frame choose to delay child-birth, and as a consequence the typical age of women presenting at an infertility clinic is 34 years. These women may have been capable of unassisted pregnancy in the past,

but due to the age-related decline in oocyte quality, they have difficulty achieving pregnancy in their 3rd and 4th decade of life. Currently, we are limited to using higher doses of gonadotropins as our main therapy for such women. As we improve our understanding of genetic and molecular changes that occur over time, we may have opportunities to intervene with older oocytes to increase the birth-rates of healthy children in this population.

CONCLUSION

Pharmacologic advances have enabled improved success in the field of reproductive endocrinology through manipulation many aspects of the menstrual cycle and early pregnancy. As molecular biologic techniques improve and individual differences between patients are discovered, pharmacologic therapies can be more highly tailored towards individual needs. Through constant collaboration and communication, pharmacologists, endocrinologists, embryologists, and physicians can continue to make continued progress in the arena of reproductive biology.

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FOOTNOTES

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FIGURE LEGENDS

Figure 1

Time line of major landmarks in the field of patient care involving in vitro fertilization. While early advancements were sparse, the previous 20 years has been fruitful for improving outcome for infertile couples. Each of these landmarks would not have been possible without the many discoveries leading to the application of the new technology highlighted.

Figure 2

Model for the hormonal feedback in the hypothalamic-pituitary-gonad axis. Hypothalamic pulsatile release of GnRH stimulates the release of FSH and LH which interacts with the ovary to stimulate both oocyte maturation, estradiol and progesterone production (see text). Estradiol and progesterone interact at the pituitary to decrease FSH and LH production.

Figure 3

Amino acid sequence of gonadotropin releasing hormone. (A) Human GnRH. "Pyro" indicates that water has been removed. Protection of terminal ends is one mechanism to increase half life. (B) Alterations in sequence that inhibit degradation and increase half-life. (C) Sensitive sites in GnRH antagonists that allow for receptor interaction without activation.

Table 1. Various Formulations of Commercially-Available Medications Used for In Vitro Fertilization

PREPARATION	TRADE NAME
Human menopausal gonadotropin (hMG)	Humegon®, Menogon®, Pergonal®, Repronex®
Purified hMG	Menopur®, Merional®
Human chorionic gonadotropin (hCG)	Novarel®, Pregnyl®, Profasi®
Recombinant hCG	Ovidrel®
Purified urinary FSH	Metrodin®, Normegon®, Orgafol®
Highly purified urinary FSH	Bravelle®, Fertinex®, Metrodin HP®
Recombinant FSH	Follistim®, Gonal-F®, Puregon®
Recombinant LH	Lhadi®, Luveris®
GnRH agonist	Decapeptyl®, Lupron®, Suprefact®, Synarel®, Zoladex®
GnRH antagonist	Antagon®, Antide®, Cetrotide®, Decapeptyl®, Nal-Glu, Plenaxis®, Teverelix,
Progesterone	Crinone®, Prometrium®, Utrogest®, Utrogestan®

Figure 1

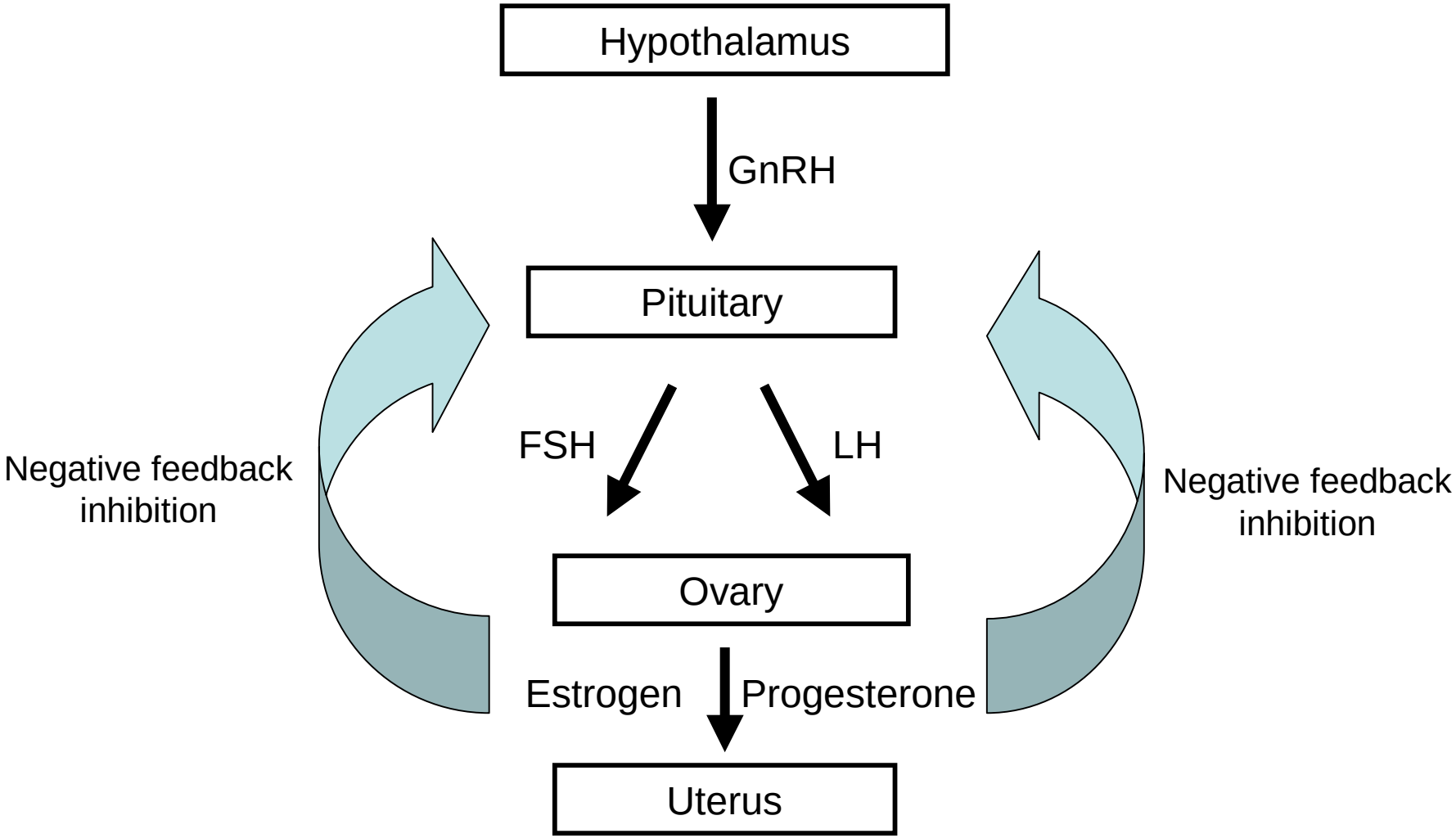

Figure 2

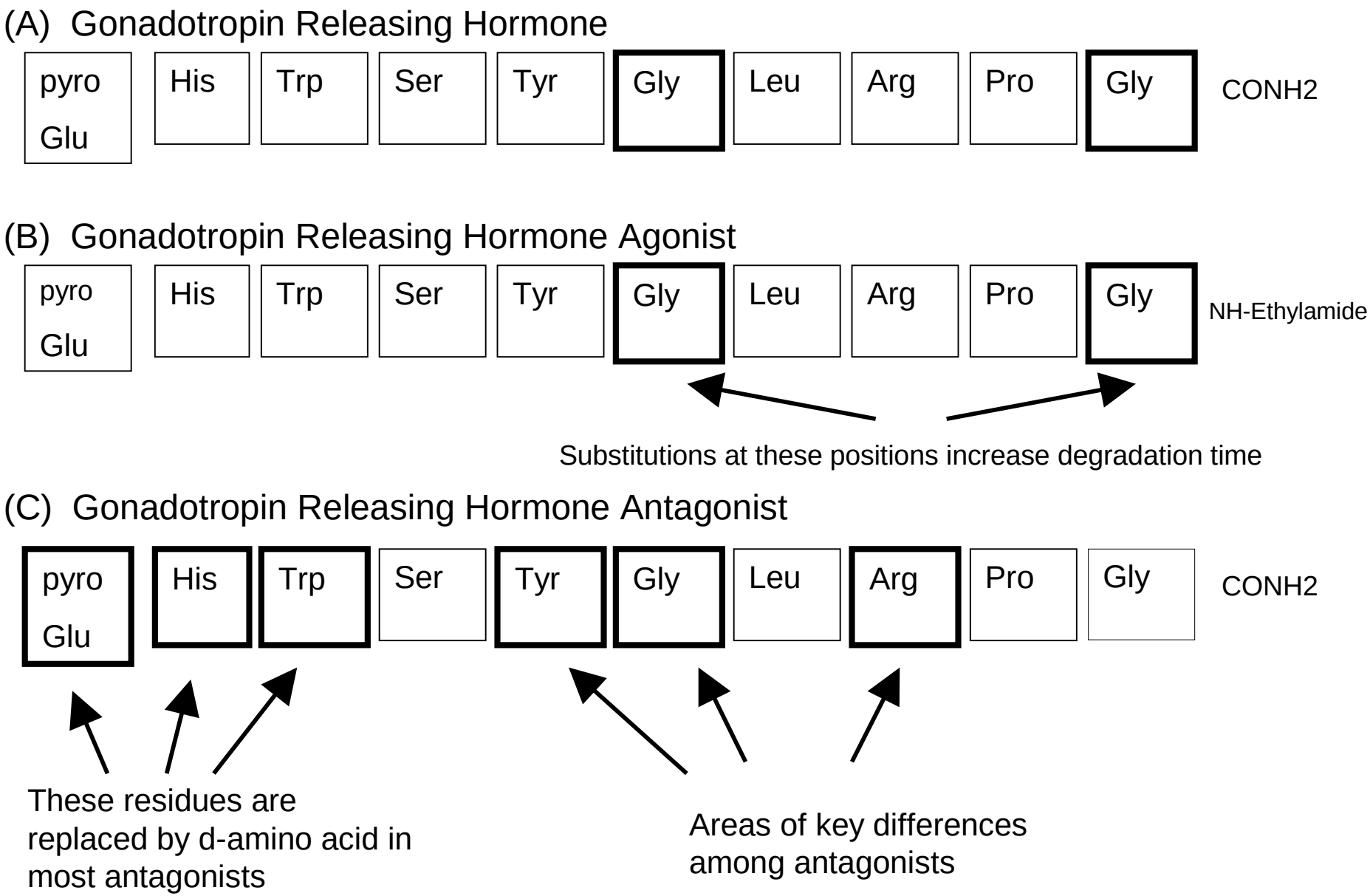

Figure 3