Review: Follicular Waves in the Human Ovary: A New Physiological Paradigm for Novel Ovarian Stimulation Protocols

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Follicular Waves in the Human Ovary: A New Physiological Paradigm for Novel Ovarian Stimulation Protocols

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Abstract

Ovulation induction (OI) is a cornerstone of human assisted reproduction treatments (ART). Current OI protocols are based on the human follicular dynamics model known as propitious moment theory (PMT), by which follicles continuously grow from the primordial pool without any pattern, and follicular fate depend on the occurrence of a gonadotropin surge. Recently, a new paradigm of human follicular dynamics called follicular waves was revealed using sequential ultrasound examination of 1 interovulatory interval. Instead of random growth, follicles develop in coordinated groups or waves, occurring 2 to 3 times during an interovulatory interval. Follicular waves are common in several other mono-ovulatory species, like equines and bovines. In fact, this model was applied to the development of several OI protocols in veterinary medicine, especially in cows. It has been shown that synchronization of OI with the emergence of a follicular wave increases substantially success rates in animals, even with single embryo transfer. Veterinarians have already developed mechanisms to control wave emergence through mechanical or chemical ablation of the dominant follicle or corpus luteum. Considering the follicular dynamics similarities between humans and bovines regarding the follicular wave phenomenon, we hypothesize that synchronization of follicular wave emergence with ovarian stimulation produces more competent oocytes and embryos and will enhance ART efficiency in humans. At the end of this article, we propose 2 theoretical approaches to induce the emergence of a follicular wave in women: (1) a mechanical strategy by aspiration of the dominant follicle and (2) a pharmacological strategy by administering estradiol and progesterone.

Keywords

follicular waves, follicular dynamics, follicular wave emergence, controlled ovarian stimulation

Introduction

The use of assisted reproduction treatments (ART) to achieve parenthood has become increasingly prevalent in the last 3 decades. One of the main strategies to augment the odds of pregnancy is increasing the number of mature oocytes through ovulation induction (OI).[1] Therefore, developing OI protocols using exogenous gonadotropins could substantially improve the success rates of low- and high-complexity ART, making OI a cornerstone of the therapy.

The development of OI protocols will require understanding ovarian follicular dynamics to enhance good quality oocyte recruitment and retrieval. Achieving the latter would ultimately allow better fertilization, embryo development, and pregnancy. Currently, all OI protocols for human ART are based on the follicular dynamics model known as propitious moment theory (PMT).[2-4] Briefly, antral follicles continuously grow from the ovarian primordial follicle pool to become gonadotropin sensitive without a clear pattern. A gonadotropin surge determines the fate of the follicle, either ovulation or atresia.[2,3] Thus, follicles that are sensitive to gonadotropin at the onset of the follicle stimulating hormone (FSH) surge will continue to grow and eventually ovulate; the nonsensitive ones become atretic.[2,3]

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A new paradigm for human follicular dynamics called follicular waves is emerging from veterinary medicine.[4,5] Follicular waves are characterized by periodic growth of a cohort of small antral follicles. There are 2 patterns of follicular waves: major and minor.[6] Both patterns share the same initial phase called emergence, during which a small group of antral follicles synchronously grow at similar rates. In the major waves pattern, a single leading follicle becomes dominant after a few days (approximately 2), continues to grow (deviation phenomenon), and is eventually ovulated, while the remaining subordinated follicles undergo atresia. In the minor waves pattern, deviation does not occur and all follicles undergo atresia. Periodic and synchronous growth of ovarian follicles is a conserved feature among a wide variety of mono-ovulatory domestic and wild animals.[7-13]

Because current OI protocols are based on the premise of PMT, the emergence of a new paradigm of follicular dynamics in humans may raise interest in new concepts for OI.

Follicular Waves in Cattle and Women

The bovine estrous cycle has 4 phases: (*a*) metaestrous, which is the period of corpus luteum formation with a gradual increase in serum progesterone concentrations; (*b*) diestrus, during which the structural and functional maturity of the corpus luteum is established via high serum progesterone (plateau); (*c*) proestrus, which is the period of functional and structural luteolysis accompanied by a sharp decrease in progesterone levels and a peak of estradiol production; and (*d*) estrus, which is marked by estrogen-mediated sexual receptivity.[14]

Ultrasound observations in cows have revealed subjects with 2 and others with 3 follicular waves in an interovulatory interval of the estrous cycle.[6] The most common pattern of follicular dynamics is an initial minor wave followed by a major wave, and then finally the last follicular wave is ovulatory.[6]

Wave emergence is preceded by an increase in endogenous FSH, which promotes the initial growth of the small antral follicles, conducive to the production of estradiol.[15] Increasing serum levels of estradiol during follicle development ultimately cause a reduction in pituitary FSH release. However, small and relatively steady concentrations of luteinizing hormone (LH) are detected throughout all follicular growth phases and contribute to final follicular development. Deviation and further growth of the dominant follicle relies on the capacity of such follicles to express LH receptors. As FSH concentrations fall, LH-sensitive follicles continue to grow and eventually undergo LH-induced ovulation, while follicles with a low density of LH receptors do not continue to grow under FSH deprivation and become atretic. This paradigm is the currently accepted mechanism for deviation.[6]

Progesterone exerts negative feedback on serum LH concentrations. Follicles that become LH sensitive when progesterone concentrations are high, as during metaestrus or diestrous, do not deviate because they are deprived of LH. They become atretic and constitute minor waves. However, follicles growing during proestrus (low progesterone concentrations) deviate, become dominant, and are ovulated under the influence of LH.[6]

Recently, follicular waves in women were characterized by daily transvaginal ultrasound examinations of the ovaries during the interovulatory interval. Similar to the bovine ovaries, those of humans have 2 or 3 follicular waves that are combinations of minor and major (Figure 1). The most frequent (75%) pattern in the human ovary is 2 waves, usually in a minor–major arrangement.[4,16]

The human menstrual cycle is essentially divided into luteal and follicular phases. The latter phase is marked by an increase in estrogen concentrations with the dominant follicle responding to a final LH peak that triggers ovulation. Luteal activity begins just after ovulation, lasts 14 days, and is followed by steroid hormone deprivation and menstruation.[17] Although the human menstrual cycle is hormonally different from the bovine estrous cycle, the fate of the emerging follicles is orchestrated mainly by gonadotropins and steroid hormones in a similar manner to cows[4-5,15] and other species.[10,18-20]

In women with two follicular waves, the first follicular wave emerges 1 day after ovulation. At this time, the high progesterone environment reduces LH pulses blocking further growth of the dominant follicle. This occurrence explains why the first wave is frequently a minor wave during which all follicles become atretic. In women with a 2-wave pattern, the second wave emerges around the time of menses, is accompanied by low serum levels of progesterone, and is associated with an increase in frequency and amplitude of LH pulses. These events lead to the deviance of a dominant follicle and its growth until ovulation occurs.[4-5]

Ovarian Stimulation in Cows

The purpose of ART in cows is to obtain an elevated number of calves from high genetic value animals. Because cows are uniparous and their gestational course is lengthy, natural conception would yield few valuable animals and selection would be extremely slow. Thus, cows are stimulated to produce a greater number of embryos that are transferred to recipient cows to deliver several newborns of superior productive value.[21]

Several strategies were developed to control the cow's ovarian function depending on the ART to be used. The objective of theriogenologists is to induce a greater number of competent oocytes by initiating OI at an appropriate time in the follicular wave. Starting stimulation at the expected time of the follicular wave produces a greater number of mature follicles,[22] decreases the doses of gonadotropins used in OI, and increases oocyte quality and embryo implantation compared to stimulation beginning in other phases.[23]

It is a common practice to control wave emergence in cows by synchronizing OI with the beginning of a wave. The hormones of the dominant follicle inhibit FSH release and its removal causes endogenous FSH to rise followed by the emergence of a new follicular wave. Therefore, ablating the dominant

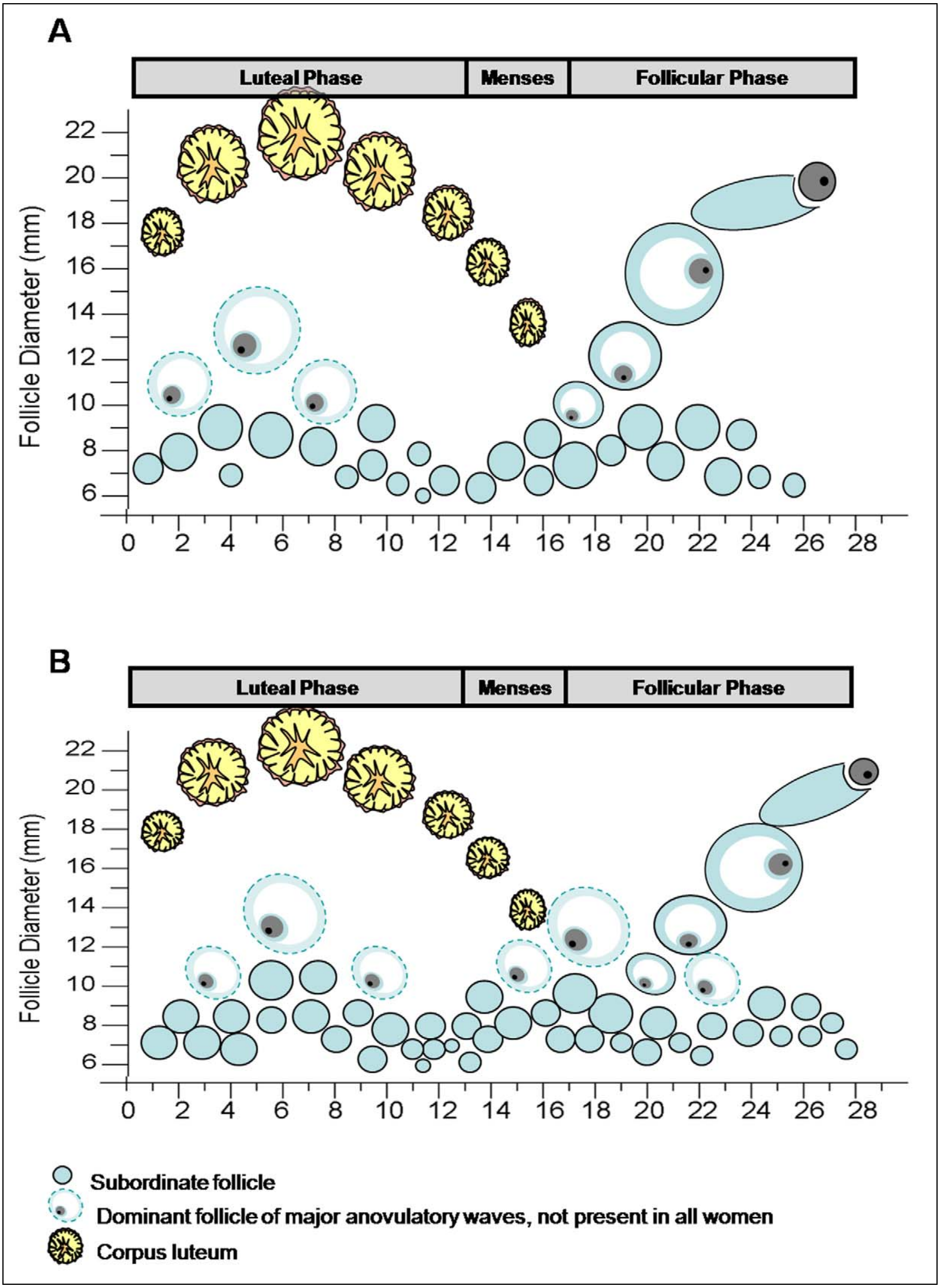

Figure 1. Emergence and regression of follicular waves in women with 2 follicular waves (A) or 3 follicular waves (B) per menstrual cycle (adapted, with permission, from Baerwald et al. 2003, Biology of Reproduction 69; p 1023-1031 and from Baerwald AR, Animal Reproduction 2009; 6(1):20-29)48.

follicle, using a transvaginal ultrasound-guided approach, is the easiest way to control wave emergence. However, this strategy is not common because it is an invasive and time-consuming procedure.[24]

Another well-known method of controlling the estrous cycle is a pharmacological one using sex steroid hormones.[25] Modern protocols for the control of wave emergence include priming with progesterone followed by a boost of estradiol.[26,27] It has been shown that a peak-like FSH release occurs with the metabolic decay of exogenous estradiol, which prompts a new wave to emerge.[26] This protocol allows the stimulation protocol to begin anytime during the estrous cycle (21-day interval).[24]

Follicular wave emergence can also be mediated by administering gonadotropin releasing hormone (GnRH), but initial results using this method were inferior to those obtained when estradiol and progesterone were used.[28] However, recent results using GnRH in cattle were encouraging.[29] Briefly, this protocol entailed inserting a controlled progesterone-releasing device into the cow's vagina, then administering GnRH 3 days later to induce ovulation. This event is followed by a rise of endogenous FSH, which recruits a new follicular wave. Ovulation induction would begin on the fifth day of treatment.

Ovulation can also be induced in cows by gonadotropin administration at the beginning of the first follicular wave,[23,30] which emerges precisely 1 day after ovulation.[31] The emergence of the first wave can usually be induced by several estrous/ovulation synchronization protocols, to avoid time-consuming heat detection. The most profitable and feasible approach is an estradiol-progestin protocol with LH-induced ovulation that can be initiated on any given day of the estrous cycle.[24]

Hypothesis

Our hypothesis is that synchronizing follicular wave emergence with ovarian stimulation will produce more competent oocytes and embryos, thereby enhancing ART efficiency in humans.

There are several lines of evidence that support this hypothesis. First, the follicular wave phenomenon in development is documented both in women and cows.[5,16] Second, the pharmacological control of follicular wave emergence can be performed in numerous animal species.[32-34] Finally, synchronization of follicular wave emergence and ovarian stimulation in animal models yields highly competent oocytes and embryos and increases implantation rates and ultimately offspring.[23]

New OI Protocols: Proposals for the Synchronization of Follicular Wave Emergence to Gonadotropin Administration in Women

Inspired by the above hypothesis, our group has proposed 2 different strategies for synchronizing follicular wave emergence to OI protocols in humans. These strategies will be discussed below as well as their implications on ART.

Mechanical Strategy—Aspiration of the Dominant Follicle

According to animal models, aspiration of the dominant follicle would be the easiest way to delete dominance and produce the emergence of a new follicular wave.[24] Dominant follicle ablation by ultrasound-guided aspiration should be an easy and feasible approach to provoke a new wave emergence in women.

There are some peculiarities about the time of the procedure's execution and the woman's ovarian cycle that should be considered before follicular aspiration. Animal models had taught that the size of the dominant follicle and the duration of its influence on the subordinate follicles interfere with wave emergence that is triggered by dominant follicle ablation.[32,35] If aspiration of dominant follicles occurs shortly after dominance is established, the deviation of the larger subordinate follicle is induced and consequently a new dominant follicle is created without emergence of a new wave. Conversely, if the ablation of the dominant follicle is postponed a few days, all subordinate follicles are irreversibly atretic due to time-dependent FSH deprivation; a new wave emergence will occur in 2 days.[32] Using daily transvaginal sonography to determine the exact time to perform dominant follicle aspiration in women may be inconvenient and impractical. Nevertheless, the time of follicular ablation in women can be planned based on knowledge of follicular emergence and growth rate.

Seventy percent of women have a 2-wave pattern of follicular development in an interovulatory interval and the remaining have a 3-wave pattern.[5] The second follicular wave emerges one day after menses in women with two-wave pattern[5] and one day before menses in those with a 3-wave pattern. Given that deviation occurs when 1 follicle reaches 10 mm in diameter and that the mean growth rate of human follicles in normoovulatory women is 1.4 mm/d,[5] follicular ablation performed on the sixth or seventh day of the menstrual cycle should remove a dominant follicle in adequate time to trigger the emergence of a new wave 2 days later. At this time, gonadotropin stimulation should be started (Figure 2).

Gonadotropin administration timed to the emergence of a follicular wave after aspiration of the dominant follicle might be 1 innovative approach for OI in humans; however, 2 mains concerns may arise: technical feasibility and patients' comfort/risk. Aspiration of the dominant follicle in a fixed time, namely in the sixth or seventh day after beginning of the menses, followed by the start of ovarian stimulation 2 days later dismisses repeated transvaginal sonography for the determination of the suitable day for gonadotropin commencement, making the procedure technical feasible. Furthermore, transvaginal follicular aspiration can performed under several types of protocols of analgesia or anesthesia for patients' comfort[36]; and it is considered a safe procedure if properly performed following safety guidelines.[37] Thus, fixed time ablation of the dominant follicle probably is a reproducible procedure in different reproductive medicine services worldwide.

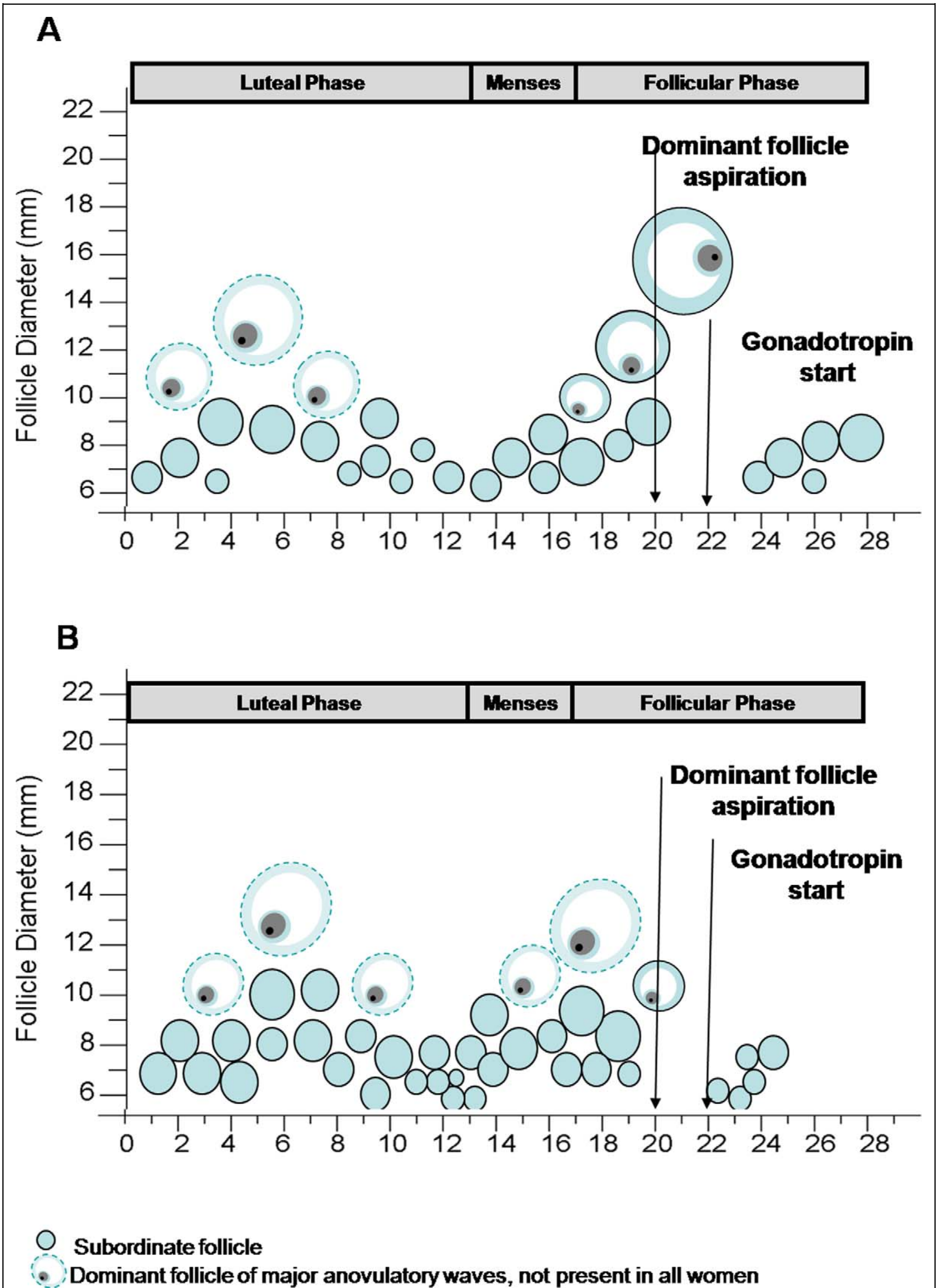

Figure 2. Mechanical strategy for synchronization of follicular wave emergence to gonadotropin administration. A, The expected effect of dominant follicle aspiration in women with 2 follicular waves per menstrual cycle. B, The expected effect of dominant follicle aspiration in women with 3 follicular waves per menstrual cycle (modified from Baerwald et al. 2003, Biology of Reproduction 69; p 1023-1031 and Baerwald AR, Animal Reproduction 2009; 6(1):20-29) 48.

Pharmacological Strategy—Estradiol and Progesterone Administration

Induction of follicular emergence with hormones administration has a tremendous advantage over other methods in that it can induce emergence anytime in the ovarian cycle of cows without loss in ART outcomes.[24] Because the control of the follicular dynamics in women and cows are similar, comparable approaches may be reliable.

In cows, one common approach is to administrate 5 mg of 17β-estradiol with 100 mg of progesterone intramuscularly at the moment of insertion of a controlled release device impregnated with 1.9 g progesterone into the vagina. Gonadotropin administration begins 4 days later. This protocol is designed to be efficient in any phase of the cow's estrous cycle[24] and its principles might be transposed to human assisted reproduction.

Treatment of women with an estradiol boost followed by a short course of progesterone administration during the follicular phase might block LH release, and lower FSH levels cause atresia of all growing follicles and loss of dominance. Conversely, if the same protocol is used during the luteal phase, it might reduce LH levels provoking luteolysis; also, estradiol has a direct luteolytic effect.[38] In either phases, after the loss of dominance or luteolysis, the pituitary surge of FSH following the metabolization of administered estradiol might induce a follicular wave emergence (Figure 3).

The development of a precise pharmacological protocol for the synchronization of follicular wave emergence to gonadotropin administration for humans demands practical experimentation and adjustments to determine adequate doses, administration route, interval, and duration. One empirical proposition could be the injection of 8 mg of estradiol valerate with daily injections of 100 mg of progesterone in oil for 5 days. The emergence of a new follicular wave might be expected to occur on the fifth day of the treatment, because the half-life of estradiol in women is short,[39] and after levels in the bloodstream fall, an FSH surge is triggered. Therefore gonadotropin administration should start on the fifth day of treatment to be synchronized with wave emergence.

Although protocols with oral contraceptive pills are currently widely used, some differences with the protocol proposed above should be emphasized. Oral contraceptive pills use a continuous combination of progesterone and low doses of estradiol for 21 days to prevent ovulation. Because most of the oral contraceptive pills used nowadays contain small doses of steroids, its efficacy decreases if started long after the beginning of the menstrual cycle. Oral contraceptives are currently used in ART cycles to avoid corpus luteum formation and to schedule patients but probably do not have an effect on the follicular wave dynamics. Conversely, high single doses of estradiol followed by a short course of progesterone are intended to produce loss of dominance/luteolysis at any time during the cycle, followed by a surge of FSH capable of initiating a follicular wave emergence. The estradiol dose required to pharmacologically induce luteolysis and elevate FSH levels followed by wave emergence in women is not known. However, administering estradiol (4 mg) should be sufficient to increase serum estradiol levels similar to those observed during the late follicular phase of the menstrual cycle. However, an estradiol-induced peak in LH levels would also be observed and it should be prevented by administering a single dose of progestin beforehand. This steroid combination should allow wave emergence to be synchronized with the start of gonadotropin administration.

Gonadotropin Dose

In cows, the synchronization of wave emergence with the start of gonadotropin administration yields a greater number of follicles for transvaginal ultrasound-guided ovum pickup.[23] Additionally, OI is accomplished with lower doses of bovine FSH.

Augmenting the number of available follicles should be beneficial for women that have a low response to OI. However, women with a typical response to gonadotropin administration should be carefully monitored for the number and size of follicles so that dose adjustments can be performed and an excessive response that could lead to the ovarian hyperstimulation syndrome can be avoided. Although, oocyte quality is not diminished by this syndrome,[40] it produces discomfort and can evolve into a serious condition that requires intense medical care.[41]

Impact of New OI Protocols on ART

The natural pregnancy rate in dairy cows is approximately 20% per estrous cycle,[42] which is similar to the incidence of natural pregnancy in humans (20% per menstrual cycle). Notwithstanding, ART in women (30% of pregnancy)[1] is less efficient than in cows (up to 68% with single embryo transfer).[43] However, there is a remarkable difference between the 2 procedures, cow embryos are usually transferred to healthy and fertile recipients after special preparation of the endometrium and human embryos are usually selected during in vitro cultivation and transferred to the same patient 3 or 5 days after OI.

Our theoretical proposals to synchronize follicular wave emergence to the start of gonadotropin treatment will not change the nature of the recipients. However, the endometrium of patients submitted to conventional OI protocols are known to have important subcellular changes with potential clinical impacts[44-46]; thus, we foresee that, if put into practice, synchronization of OI with follicular wave emergence may cause premature exposure of the endometrium to high progesterone levels, which might interfere with adequate endometrial development and receptivity.

Consequently, one might anticipate that transference of fresh embryos after OI synchronized with wave emergence should be avoided; and, therefore, embryos must be cryopreserved by vitrification to accommodate endometrial preparation and embryo transfer to a more favorable uterine environment. Thence, if the proposals for the synchronization of follicular

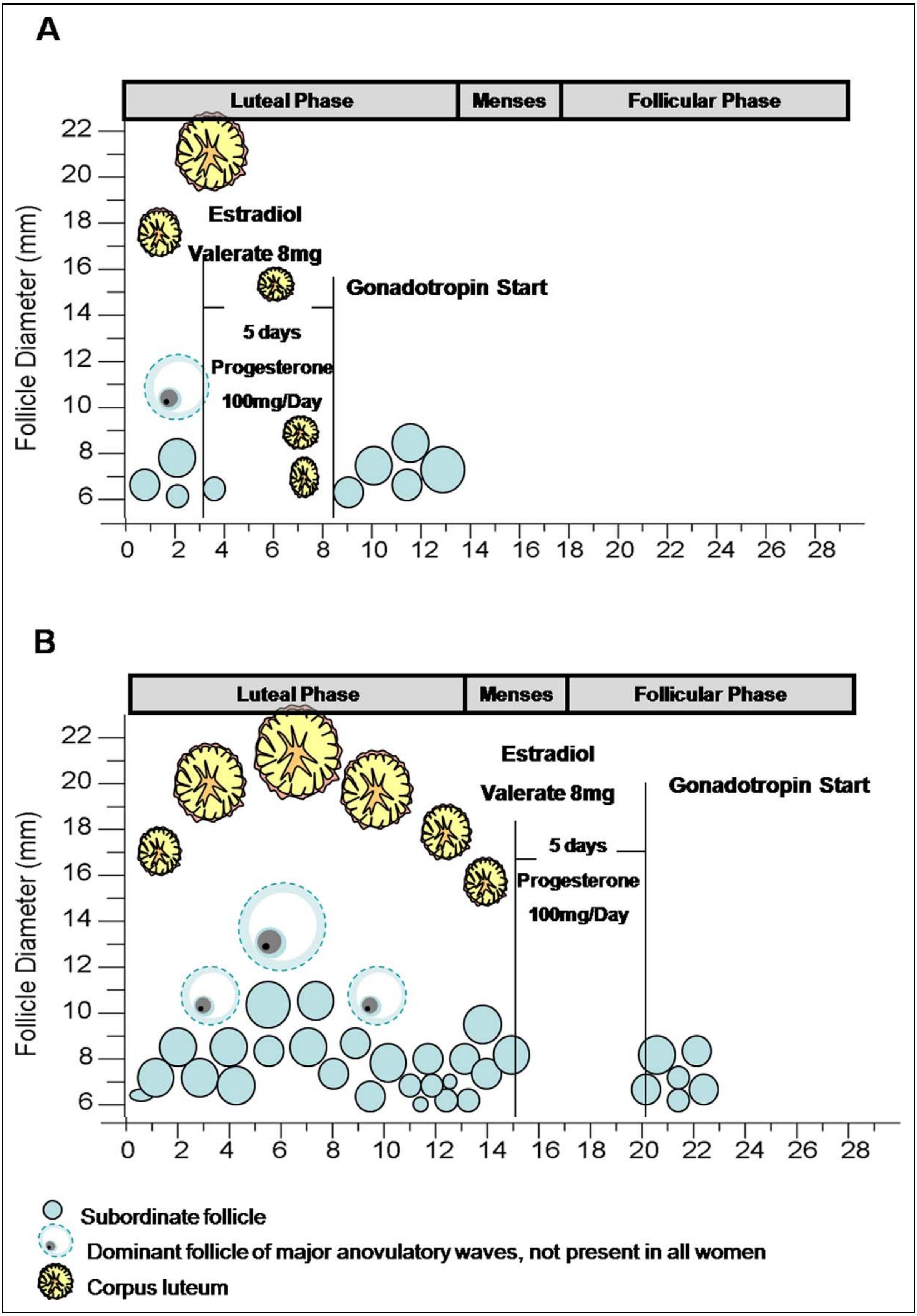

Figure 3. Pharmacological strategy for synchronization of follicular wave emergence to gonadotropin administration. A, The expected effect of the pharmacological protocol started during the luteal phase of the menstrual cycle. B, The expected effect of the pharmacological protocol started during menses. C, The expected effect of the pharmacological protocol started during the follicular phase of women with 2 follicular waves. D, The expected effect of the pharmacological protocol started during the follicular phase of women with 3 follicular waves (modified from Baerwald et al. 2003, Biology of Reproduction 69; p 1023-1031 and Baerwald AR, Animal Reproduction 2009; 6(1):20-29) 48.

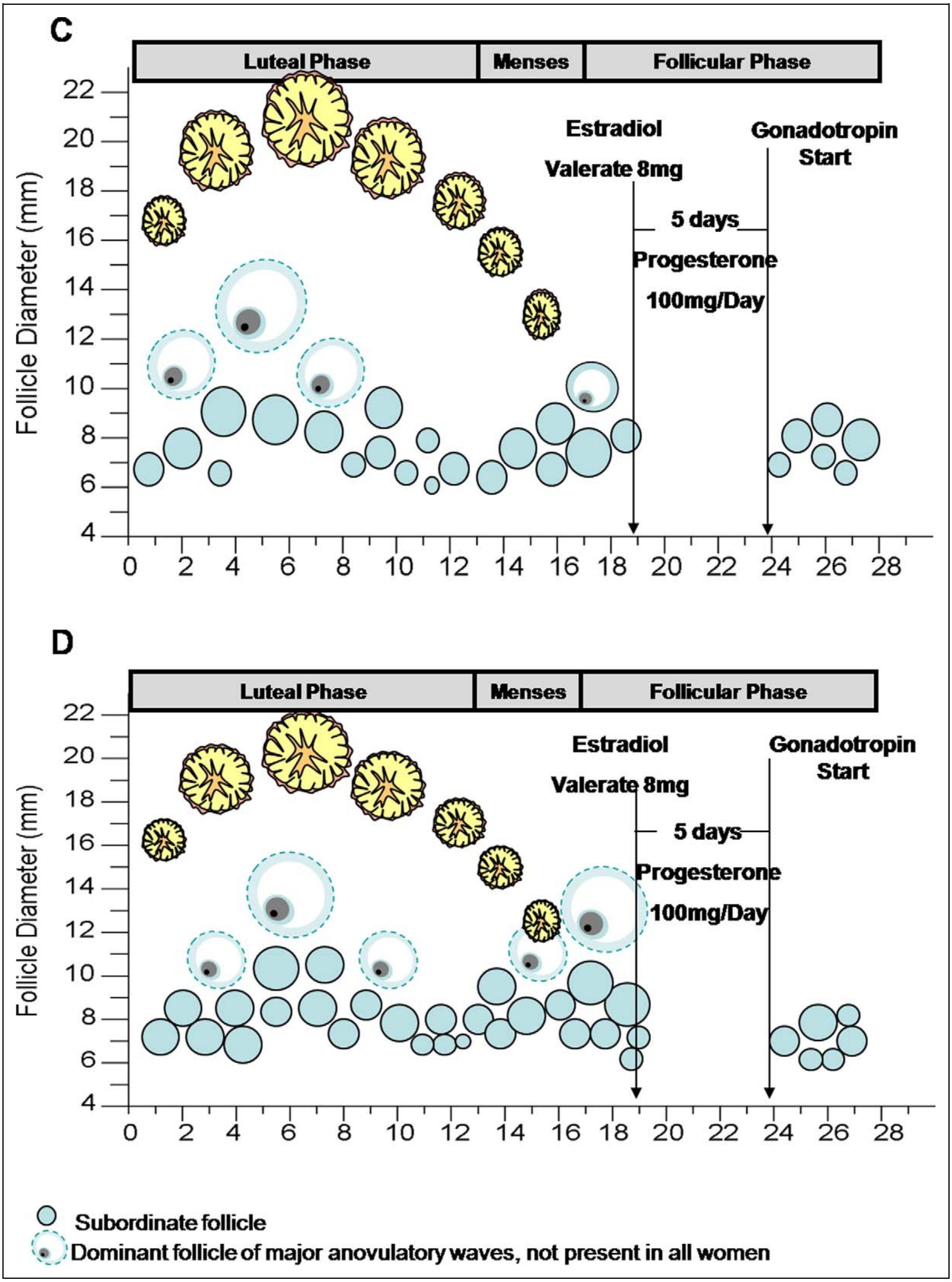

Figure 3 continued

wave emergence to gonadotropin administration described in this manuscript get proved, in vitro fertilization (IVF) programs adopting them should consider improving their embryologist's skills in cryopreservation of oocytes and embryos.

If our assumptions are right, one possible impact on OI might be lowering the requirements of FSH and reduction of medication expenses that might increase the cost/benefit rate of ART and promote a greater number of patients adhering to treatment. Additionally, if the benefits of the synchronization of follicular wave emergence to gonadotropin administration are confirmed, one might speculate that the rate of pregnancy in humans may reach that seen in cows after single embryo transfer; therefore, a greater number of patients could be assigned to single embryo transfer programs contributing to a significant reduction in multiple pregnancies. Furthermore, the number of surplus, cryopreserved embryos of superior quality that are available for future transfers would be increased and the cumulative pregnancy rate per OI cycle would rise.

The experimental designs for developing protocols for follicular wave synchronization are being evaluated, and hopefully, beneficial treatments using this concept will arise in the following years to aid patients undergoing ART to achieve parenthood.

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