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Mitri F, Behan LA, Murphy CA, Hershko-Klement A, Casper RF, Bentov Y.
Microdose flare protocol with interrupted follicle stimulating hormone and added androgen for poor responders-an observational pilot study.
Fertil Steril. 2016 ;105:100-105.

RESUMEN ORIGINAL

Objective: To investigate whether temporarily withholding FSH and adding androgen could improve follicular response during a microdose flare protocol in women with slow follicular growth or asynchronous follicular development.

Design: Observational pilot study.

Setting: University-affiliated private fertility center.

Patient(s): Twenty-six women aged 34-47 years with poor response to stimulation or a previous cancelled IVF cycle and with slow or asynchronous follicular growth during a microdose flare cycle.

Intervention(s): For 13 women, after initiation of ovarian stimulation using the microdose flare protocol, gonadotropin administration was interrupted and transdermal testosterone gel was added for several days (4.4 ± 1.2 d) starting after cycle day 7 (mean cycle day 10 ± 2.6).

Main outcome measure(s): FSH, E2, follicular growth, and total number of mature oocytes retrieved were determined for all of the patients. Cycle cancellation rate as well as pregnancy rate following embryo transfer were also documented when applicable.

Result(s): FSH levels declined (25.2 ± 6.5 to 6.8 ± 3.2 IU/L), E2 levels increased (896 ± 687 to 2,163 ± 1,667 pmol/L), and follicular growth improved significantly during gonadotropin interruption and were tracked for 2 days during this time frame. The average number of oocytes retrieved was 5.3 ± 2.6, and the ratio of mature to total oocytes was 4:5. Four of the 13 women in the interruption group conceived following frozen embryo transfer, whereas none in the control group did.

Conclusion(s): The androgen-interrupted FSH protocol may improve follicular response to gonadotropins in cycles that might otherwise be cancelled.

TRADUCCIÓN Y COMENTARIOS

Introducción

Los autores comienzan destacando la complejidad de las pacientes que responden subóptimamente a la estimulación ovárica. La categorización de tales pacientes como de pobres respondedoras no solo carece de consenso unánime, existiendo en la actualidad más de 35 definiciones (1), sino que constituyen un grupo de difícil tratamiento debido al bajo número de ovocitos y embriones obtenidos y para el que los numerosos tratamientos propuestos no parecen haber añadido beneficio alguno (2).

Citan los autores un reciente metanálisis de Bosdou (3) en donde se concluye que la testosterona transdérmica puede mejorar embarazo clínico y las tasas de nacimientos vivos en las pobres respondedoras. La justificación para este tratamiento se basaba en estudios realizados en primates que demuestran que el aumento de los niveles intrafoliculares de andrógenos pueden regular al alza los receptores de FSH en las células de la granulosa, mejorar la respuesta a las gonadotropinas y aumentar el crecimiento folicular (4).

Sin embargo, frente a esta base fisiopatológica para mejorar la respuesta folicular, es frecuente observar como en la práctica clínica se emplean grandes cantidades de gonadotropinas basadas solo en hábitos o reglas arbitrarias que no mejoran ni la respuesta folicular, ni el rendimiento de los ovocitos, ni el resultado global (5, 6).

Un punto fuerte de este artículo radica en que esos mismos autores habían demostrado previamente (7) que niveles séricos de FSH de > 20 UI / L en el día 7º del ciclo (día 4º de estimulación) podrían indicar saturación del receptor de FSH en las células granulosa y que, por lo tanto, la adición de más FSH exógena no resultaría en un aumento de la respuesta folicular.

Otro aspecto importante, referido por los autores, es que los folículos antrales, especialmente los de pacientes con respuesta deficiente, expresan un amplio espectro de sensibilidades a la FSH que podría ocasionar un crecimiento monofolicular o asincrónico, a pesar de una cohorte amplia de pequeños folículos antrales al comienzo del ciclo (8).

El objetivo de este estudio observacional fue determinar si la retirada de las gonadotropinas y la administración de suplementos de testosterona transdérmica, cuando se había observado una pobre respuesta, podría sensibilizar y sincronizar el crecimiento folicular.

MATERIAL Y MÉTODOS

Población de estudio

Las 13 mujeres incluidas en este estudio piloto observacional en The Toronto Centre for Advanced Reproductive Medicine (TCART) , entre octubre de 2013 y diciembre de 2014, tenían por lo menos un ciclo anterior de FIV fallida o cancelada, y sospecha de resistencia a la gonadotropina (FSH ≥20 suero mUI / L en el día 7), conjuntamente con un crecimiento folicular ausente o mínimo durante el ciclo actual (definido como aumento de <0,5 mm por día de diámetro promedio después de 5-8 días de estimulación con altas dosis.

Seis de estas pacientes habían confirmado una respuesta deficiente en base a los criterios de Bolonia. El resto de las mujeres habían fallado ciclos anteriores de FIV, y se esperaba que algunas de ellas fueran pobres respondedores.

Grupo control

Este grupo estaba constituido por 13 pacientes tratadas entre abril de 2006 Noviembre de 2011, antes de que el uso de gel tópico de testosterona se usara en esa clínica. Los casos elegidos tenían que incluir un conjunto de datos que evidenciaran un nivel de FSH en suero sugestivo de resistencia a gonadotropina.

Los ciclos de FIV se hicieron en protocolo de micro-flare y no fueron interrumpidos aunque mostraron un desarrollo folicular pobre o asincrónico.

Protocolo de estimulación ovárica

El protocolo de micro-flare se inició mediante el uso de FSH recombinante en el 3er día de la menstruación (Gonal F, EMD Serono, o Puregon, Merck). Cinco mujeres recibieron 150 IU/12 horas y ocho mujeres 200 UI/12 horas de FSH recombinante de inicio a partir del día 3 del ciclo. Las concentraciones séricas de FSH y E2 fueron medidas por inmunoensayo con el uso del Sistema de Vitros ECiQ inmunodiagnóstico (Ortho-Clinical Diagnostics, Johnson y Johnson). Los niveles basales necesarios para el inicio de la estimulación fueron : FSH ≤15 UI / L y E2 <200 pmol / L, de lo contrario el ciclo fue cancelado.

El agonista de la GnRH empleado fue acetato de buserelina (Suprefact; Sanofi-Aventis) que se administró a una dosis de 50 mg por vía subcutánea dos veces al día, a partir del día 3. Los niveles séricos de FSH, LH, P, y E2 fueron medidos al inicio del estudio y en cada visita en todos los pacientes. Cuando los niveles séricos de FSH superaron 20 UI / L en el día 7º del ciclo, o en cualquier otro momento a partir de entonces, o el crecimiento folicular se hizo lento o asíncrono, se interrumpieron las gonadotropinas durante 4-7 días.

A los pacientes se les dio la opción de volver a la clínica en cualquier momento durante este período de tiempo y las gonadotropinas se reiniciaron inmediatamente a la misma dosis.

La administración de 25 mg de testosterona transdérmica diaria (2,5 g 1% Androgel; Abbott) comenzó el día en que las inyecciones de FSH fueron interrumpidas y finalizó el día en que se desencadenó la ovulación. Este es uno de los tratamientos estándar se ofrecen en el TCART a los pacientes que responden de la forma descrita anteriormente.

El crecimiento folicular se considera que es asincrónico cuando uno o dos folículos principales eran ≥4 mm mayor (diámetro promedio) que el resto de la cohorte.

El protocolo de estimulación para los pacientes que se incluyeron en el grupo de control no incluía la retirada del tratamiento con gonadotropinas o uso de la testosterona tópica.

Cuando se observaron al menos tres folículos dominantes y al menos dos habían alcanzado un tamaño de ≥18 mm, se administraron 10.000 UI de hCG (Pregnyl; Merck) por vía subcutánea para desencadenar la ovulación. Treinta y seis horas más tarde, los ovocitos fueron recuperados por medio de aspiración ecodirigida con aguja transvaginal. Después de la extracción ovocitaria, se realizó inyección intracitoplasmática espermática en 12 pacientes, FIV convencional en un caso , y congelación de ovocitos en tres (en total para los dos grupos se hicieron 13 inyecciones intracitoplasmáticas de esperma, 1 FIV convencional, 3 congelaciones de ovocitos y 9 conversiones a inseminación intrauterina,- un total de 26 para los 2 grupos). Todos los embriones se transfirieron en etapa de división o en blastocisto, en función del criterio del médico y del paciente, y algunos embriones fueron vitrificados.

Análisis estadístico

Se realizó con el uso de Graphpad Prism versión 5.02. Para el estudio comparativo se usaron el test de Wilcoxon para pares emparejados y el test de t pareado.

RESULTADOS

Los dos grupos fueron similares en edad, índice de masa corporal, y niveles séricos basales de FSH y E2.

Nueve de las 13 pacientes en el grupo de control tuvieron que reconvertirse en inseminación intrauterina debido a un número insatisfactorio de folículos maduros obtenidos.

En el grupo de estudio, las gonadotropinas se retiraron el día 10±2.6 del ciclo. La duración de esta suspensión de gonadotropinas osciló entre 3 y 7 días (media 4,4± 1,2 d). La determinación de FSH, E2, y tamaños de folículos se registraron en todos participantes en el primer día de la interrupción de gonadotropina y en el día de reanudación. Cuando la estimulación de gonadotropina se reanudó, los niveles de FSH habían disminuido significativamente (de 25,2± 6,5 UI /L de promedio a 6,8 ±3,2 UI/L) aproximándose a los de la línea de base. Sin embargo, los niveles de estrógeno y el número de folículos de > 1 cm aumentaron durante el intervalo de la retirada de gonadotropina, y el número promedio de ovocitos obtenidos estuvo muy cerca del de folículos de > 1,6 cm medidos en el momento de desencadenar la ovulación (5,9± 4.1 vs. 4.9± 2,7) y, para la mayoría de los pacientes, relacionado con el recuento de folículos antrales.

Se obtuvieron una proporción razonable de ovocitos maduros (4,53± 2,98 vs. 2,75± 1.3) y un total de cuatro embarazos (ninguno en el grupo control), incluyendo un embarazo gemelar, entre las diez mujeres que tenían transferencia embrionaria.

DISCUSIÓN

Los autores recuerdan que cuando no era posible el empleo de los antagonistas de la GnRH, el protocolo largo con agonistas era ampliamente utilizado en pacientes con alto riesgo de hiperestimulación ovárica, y el costing" era una salvaguardia contra OHSS. (9). Durante el coasting, por lo general, los niveles de E2 seguían aumentando durante 1 o 2 días tras la retirada de gonadotropinas, antes de descender bruscamente (10, 11). Los folículos> 1,5 cm normalmente seguían creciendo y muchos de ellos sobrevivían durante varios días en ausencia de soporte gonadotrópico (12), pero las células de la granulosa de los folículos más pequeños sufrían apoptosis y esto conducía a una atresia folicular(13).

La vida media de La FSH recombinante se estima que oscila entre 24 y 40 horas, y los niveles de estado estacionario se alcanzan después de 3-5 días de tratamiento (14).

En el presente estudio, aunque las gonadotropinas se retiraron de una de manera similar al coasting, el resultado fue claramente diferente. Los niveles de E2 siguieron aumentando y los folículos creciendo, incluso después de haber transcurrido una semana del cese de gonadotropinas, con niveles séricos de FSH significativamente más bajos en comparación con el período previo a la interrupción y el grupo control.

Para los autores, esto pone de relieve las diferencias existentes tras la retirada de gonadotropina entre las mujeres con pobre y alta respuesta.

Las hiperrespondedoras, con una gran cohorte de folículos grandes y pequeños y con un gran número de receptores de FSH, retiran rápidamente la FSH del suero cuando se hace coasting y este descenso conduce a la atresia folicular.

Por el contrario, las mujeres con pobre respuesta, al tener un número mucho menor de folículos en crecimiento, poseen un menor número de receptores de FSH y utilizan las gonadotropinas de una manera mucho más lenta. Sus umbrales son más altos para la FSH y requieren mayores niveles de gonadotropina para responder.

Esto queda reflejado en el nivel sérico elevado de FSH después de la administración de una dosis estándar de FSH y en el promedio de 5 días requerido para que los niveles séricos de FSH disminuyan a los niveles pre estimulación (7).

La constatación de que los folículos continuaban creciendo a pesar de la disminución de los niveles de FSH, les sugiere a los autores distintas posibilidades:

a) En estas circunstancias los folículos son "más eficaces" para la utilización de gonadotropinas, posiblemente como resultado del andrógeno añadido coincidente con la interrupción de la FSH.

b) Al ser menor la supresión hipofisaria con el protocolo de micro-flare respecto del protocolo largo (15-17), puede existir algún residuo de gonadotropinas endógenas que facilitase el crecimiento de los pequeños folículos antrales.

c) Existe un posible efecto estimulante directo de los agonistas GnRH hacia los ovarios ya que los niveles de E2 descienden más bruscamente durante el coasting cuando ámbos, agonista de GnRH y gonadotropinas, se retiran conjuntamente, que cuando se mantiene el agonista y sólo se retira la FSH (18, 19). Además, la caída de E2 es más rápida y la duración del coasting es generalmente más corta en los ciclos con antagonistas vs. agonistas de GnRH (19). Es posible que los agonistas de GnRH pudieran unirse a los receptores de GnRH del ovario y estimular el crecimiento folicular directamente (20).

d) Intervención de ciertos factores locales intraováricos, como el péptido inhibidor (FSHBI) que bloquea la unión de FSH a su receptor en la granulosa (21). Una administración excesiva de FSH exógena, al aumentar la producción de FSHBI, suprimiría el crecimiento y desarrollo folicular.

e) Los andrógenos son cruciales para la diferenciación y señalización de células de la granulosa, en particular durante las primeras etapas del desarrollo folicular. Pero los niveles de andrógenos intraováricos deben estar equilibrados ya que niveles excesivos pueden producir apoptosis y niveles insuficientes afectar al crecimiento y el desarrollo folicular.

Los andrógenos han demostrado que regulan al alza la expresión del receptor de FSH (4, 22) y que proporcionan un sustrato para la aromatización inducida por la FSH contribuyendo a un aumento de los niveles de E2.

Tras la interrupción de FSH, la inducción androgénica de receptores de FSH por la administración exógena de testosterona, junto con la disminución de los niveles séricos de FSH recombinante , podrían potenciarse para sensibilizar a los folículos en crecimiento y aumentar la "eficiencia" en la utilización de las gonadotropinas.

Aunque los autores reconocen que no fue posible comparar estas dos alternativas terapéuticas para cada paciente, el resultado global demuestra una mejor respuesta con la supresión de gonadotropinas y la administración de testosterona transdérmica.

Los niveles de E2 y el número de folículos > 1 cm casi se triplicaron durante el breve intervalo de interrupción de gonadotropinas y fueron significativamente mayores en comparación con el grupo control.

El número total de ovocitos obtenido se correlacionó con el recuento basal de folículos antrales.

El número de folículos dominantes (≥1.4 cm) en el momento de la inducción de la ovulación también se correlacionó con el número de oocitos maduros recuperado, sugiriendo una estimulación óptima de los folículos antrales presentes.

El consumo sérico de FSH se mantuvo durante la interrupción de las gonadotropinas y aumentó la producción de estrógenos. La testosterona transdérmica probablemente contribuyó a este mayor pico E2 sérico: a) al ser sustrato para la enzima aromatasa y b) por su papel sensibilizador en las células de la granulosa de la inducción de más aromatasa por la FSH.

Así pues, interrumpir temporalmente las gonadotropinas y suplementar con testosterona transdérmica parece ser una alternativa simple y rentable a la cancelación del ciclo. Los pacientes que requieren grandes dosis de gonadotropinas, o tienen elevados los niveles séricos de FSH (> 20 IU / L) durante la estimulación, en conjunción con un crecimiento folicular mínimo o asincrónico podrían beneficiarse de esta estrategia. Los recuentos basales elevados de folículos antrales elevados parecen correlacionarse con un mejor resultado.

Si, después de la interrupción de gonadotropinas, la respuesta se considerase subóptima, todavía podría cancelarse el ciclo sin ningún gasto adicional.

La carga emocional y psicológica de tener ciclos de estimulación cancelados no se debe subestimar, particularmente en pacientes que ya han recibido un gran número de inyecciones y han invertido altos costos en la medicación (23, 24).

Curiosamente, el crecimiento folicular se mantuvo incluso después de 7 días de retirada de gonadotropinas, aunque estudios anteriores sugieren que la interrupción no es recomendada > 5 días ya que puede ser perjudicial para los ovocitos (25).

Finalizan los autores señalando las limitaciones del estudio debidas al pequeño tamaño de la muestra, carácter retrospectivo, y múltiples intervenciones utilizadas y afirmando que se precisan estudios aleatorios más grandes para apoyar sus resultados.

REFERENCIAS CONSIDERADAS DE INTERÉS POR EL EDITOR

resúmenes de las mismas

1. Ferraretti AP, La Marca A, Fauser BC, Tarlatzis B, Nargund G, Gianaroli L. ESHRE consensus on the definition of "poor response" to ovarian stimulation for in vitro fertilization: the Bologna criteria. Hum Reprod 2011;26:1616–24.

The definition presented here represents the first realistic attempt by the scientific community to standardize the definition of poor ovarian response (POR) in a simple and reproducible manner. POR to ovarian stimulation usually indicates a reduction in follicular response, resulting in a reduced number of retrieved oocytes. It has been recognized that, in order to define the poor response in IVF, at least two of the following three features must be present: (i) advanced maternal age or any other risk factor for POR; (ii) a previous POR; and (iii) an abnormal ovarian reserve test (ORT).

Two episodes of POR after maximal stimulation are sufficient to define a patient as poor responder in the absence of advanced maternal age or abnormal ORT. By definition, the term POR refers to the ovarian response, and therefore, one stimulated cycle is considered essential for the diagnosis of POR. However, patients of advanced age with an abnormal ORT may be classified as poor responders since both advanced age and an abnormal ORT may indicate reduced ovarian reserve and act as a surrogate of ovarian stimulation cycle outcome. In this case, the patients should be more properly defined as 'expected poor responder'. If this definition of POR is uniformly adapted as the 'minimal' criteria needed to select patients for future clinical trials, more homogeneous populations will be tested for any new protocols. Finally, by reducing bias caused by spurious POR definitions, it will be possible to compare results and to draw reliable conclusions.

2. Tarlatzis BC, Zepiridis L, Grimbizis G, Bontis J. Clinical management of low ovarian response to stimulation for IVF: a systematic review. Hum Reprod Update 2003;9:61–76.

Poor response is not a rare occurrence in ovarian stimulation. Although not fully accepted, the most dominant criteria for poor ovarian response are small numbers of follicles developed or oocytes retrieved, and low estradiol (E2) levels after the use of a standard stimulation protocol. There is no ideal predictive test as the poor responder is revealed only during ovulation induction; however, increased levels of day 3 FSH and E2 as well as decreased levels of inhibin B can be used to assess ovarian reserve. Several protocols have been proposed for clinical management of low ovarian response in IVF. Although high doses of gonadotrophins have been used by the vast majority of authors, results have been controversial and prospective randomized studies have shown little or no benefit. The few available relevant studies do not indicate that recombinant FSH improves outcome. Flare-up GnRH agonist protocols (including all dosage varieties) produce better results than standard long luteal protocols. Luteal initiation GnRH agonist 'stop' protocols were shown to improve ovarian response according to prospective studies with historical controls, but this was not confirmed by well-designed prospective, randomized, controlled studies. The few available data obtained with GnRH antagonists have not shown any benefits. Adjuvant therapy with growth hormone (GH) or GH-releasing factors results in no significant improvement. The use of corticosteroids reduces the incidence of poor ovarian response in women undergoing IVF treatment. The limited data obtained with nitric oxide donors are encouraging. Pretreatment with combined oral contraceptives prior to stimulation may help ovarian response. No benefit was observed with standard use of ICSI or assisted hatching of zona pellucida. Finally, natural cycle IVF has produced results which are comparable with those obtained with stimulated cycles in true poor responders. Well-designed, large-scale, randomized, controlled trials are needed to assess the efficacy of these different management strategies.

3. Bosdou JK, Venetis CA, Kolibianakis EM, Toulis KA, Goulis DG, Zepiridis L,et al. The use of androgens or androgen-modulating agents in poor responders undergoing in vitro fertilization: a systematic review and metaanalysis. Hum Reprod Update 2012;18:127–45.

BACKGROUND The aim of this meta-analysis was to evaluate the role of androgens or androgen-modulating agents on the probability of pregnancy achievement in poor responders undergoing IVF.

METHODS Medline, EMBASE, CENTRAL, Scopus and Web of Science databases were searched for the identification of randomized controlled trials evaluating the administration of testosterone, dehydroepiandrosterone (DHEA), aromatase inhibitors, recombinant luteinizing hormone (rLH) and recombinant human chorionic gonadotrophin (rhCG) before or during ovarian stimulation of poor responders.

RESULTS In two trials involving 163 patients, pretreatment with transdermal testosterone was associated with an increase in clinical pregnancy [risk difference (RD): +15%, 95% confidence interval (CI): +3 to +26%] and live birth rates (RD: +11%, 95% CI: +0.3 to +22%) in poor responders undergoing ovarian stimulation for IVF. No significant differences in clinical pregnancy and live birth rates were observed between patients who received DHEA and those who did not. Similarly, (i) the use of aromatase inhibitors, (ii) addition of rLH and (iii) addition of rhCG in poor responders stimulated with rFSH for IVF were not associated with increased clinical pregnancy rates. In the only eligible

study that provided data, live birth rate was increased in patients who received rLH when compared with those who did not (RD: +19%, 95% CI:+1 to +36%).

CONCLUSIONS Based on the limited available evidence, transdermal testosterone pretreatment seems to increase clinical pregnancy and live birth rates in poor responders undergoing ovarian stimulation for IVF. There is insufficient data to support a beneficial role of rLH, hCG, DHEA or letrozole administration in the probability of pregnancy in poor responders undergoing ovarian stimulation for IVF.

4. Weil S, Vendola K, Zhou J, Bondy CA. Androgen and follicle-stimulating hormone interactions in primate ovarian follicle development. J Clin Endocrinol Metab 1999;84:2951–6.

We have previously shown that androgens stimulate early stages of follicular development and that granulosal androgen receptor (AR) gene expression is positively correlated with follicular growth. The present study was aimed at elucidating potential interactions between FSH and androgens in follicular development. Study groups included eight normal cycling rhesus monkeys (five follicular and three luteal-phase), eight testosterone (T)-treated, and four FSH-treated animals. Examination of sequential ovary sections revealed selective colocalization of AR and FSH receptor (FSHR) messenger RNAs (mRNAs) in healthy, growing follicles. Moreover, individual follicles demonstrate a highly significant ($P < 0.001$) positive correlation between FSHR and AR mRNA levels in all study groups. Androgen treatment significantly increased granulosa cell FSHR mRNA abundance (by approximately 50–100%, depending on follicle size). FSH treatment increased granulosa AR mRNA levels only in primary follicles. The finding that T augments follicular FSHR expression suggests that androgens promote follicular growth and estrogen biosynthesis indirectly, by amplifying FSH effect, and may partially explain the enhanced responsiveness to gonadotropin stimulation noted in women with polycystic ovary syndrome.

We have recently shown that androgens stimulate early stages of follicular growth in the rhesus monkey ovary (1, 2). Primary, secondary, and tertiary (small antral) follicles are significantly increased in number, and granulosa and thecal cell proliferation are significantly increased in T- and dihydrotestosterone-treated animals (1, 2). Furthermore, granulosa cell androgen receptor (AR) gene expression is positively correlated with proliferation and negatively correlated with apoptosis in the monkey ovary (3). Evidence from in vitro models is conflicting, with some data suggesting antiproliferative or atretogenic effects (4), whereas other data indicate that androgens promote follicular growth (5, 6). Women with hyperandrogenism have impaired ovulatory function, but this may be caused by excessive numbers of small growing follicles disrupting normal hypothalamic-pituitary-ovary interaction, as opposed to atretogenic effects by androgen. Supporting this view, ovaries from women with polycystic ovary syndrome (PCOS) have increased numbers of small growing follicles (7). Furthermore, granulosa proliferation and steroidogenesis seem robust in PCOS follicles (8, 9), and androgen blockade results in reduction in follicle number and resumption of ovulatory cycles (10).

The mechanism(s) whereby androgens stimulate follicular growth remain unclear. Because infertile women with PCOS frequently hyperrespond to FSH treatment for ovulation induction (11, 12), and granulosa cells from PCOS ovaries are hyperresponsive to FSH treatment in vitro (13), we considered the possibility that androgens might promote granulosa FSH receptor (FSHR) expression. Therefore, in the present work, we have investigated the relation between follicular AR and FSHR expression, and we examined the effects of androgens on follicular FSHR messenger RNA (mRNA) levels as well as the effects of FSH on AR mRNA levels.

5. van Hooff MH, Alberda AT, Huisman GJ, Zeilmaker GH, Leerentveld RA. Doubling the human menopausal gonadotrophin dose in the course of an in-vitro fertilization treatment cycle in low responders: a randomized study. Hum Reprod 1993;8:369–73.

The effect of doubling the human menopausal gonadotrophin (HMG) dose in the same treatment cycle in which the ovarian response after 5 days of ovarian stimulation with 225 IU/day is 'low', has been evaluated in a prospective randomized study. Forty-six patients met the ultrasound and oestradiol criteria for enrolment in the study, one patient participated twice. In 22 patients treatment was continued with 225 IU HMG/day and in 25 patients the HMG dose

was increased to 450 IU/day. No effect of doubling the HMG dose was found on the length of the ovarian stimulation, peak oestradiol values, number of follicles ≥ 11 and ≥ 14 mm in diameter respectively on ultrasound on the day of HCG administration, number of cancelled cycles, number of oocytes at follicular puncture and the number of patients with ≥ 3 oocytes at retrieval. It is concluded that doubling the HMG dose in the course of an IVF treatment cycle is not effective in enhancing ovarian response in low responders. This is in accordance with current theories on follicular growth, which state that follicular recruitment occurs only in the late luteal and early follicular phase of the menstrual cycle.

6. Karande VC, Jones GS, Veeck LL, Muasher SJ. High-dose follicle-stimulating hormone stimulation at the onset of the menstrual cycle does not improve the in vitro fertilization outcome in low-responder patients. Fertil Steril 1990;53:486–9.

I*n an attempt to improve their outcome with in vitro fertilization (IVF), 34 low-responder patients were stimulated with six ampules of follicle-stimulating hormone (FSH) daily starting on day 1 (n = 17) or day 2 (n = 17) of their menstrual cycles. The stimulated cycles showed a mean peak estradiol of 443 ± 173 pg/mL, mean days of human chorionic gonadotropin of 7.6 ± 1.4, 2.67 ± 1.5 preovulatory oocytes per retrieval, and 2.56 ± 1.3 oocytes per transfer. Three clinical pregnancies resulted after 25 embryo transfer cycles (12%). With paired analysis, we compared 8 patient cycles with prior six ampules of FSH stimulation starting on day 3; all parameters examined showed no significant differences. In a comparison of 22 patients cycles with prior 4 ampules of FSH stimulation on cycle day 3, no significant differences in any parameters were observed except in the higher number of ampules used in the present study. We conclude that high-dose FSH stimulation at the onset of the menstrual cycle does not improve the IVF outcome in low-responder patients.*

7. Bentov Y, Burstein E, Firestone C, Firestone R, Esfandiari N, Casper RF. Can cycle day 7 FSH concentration during controlled ovarian stimulation be used to guide FSH dosing for in vitro fertilization? Reprod Biol Endocrinol 2013;11:12.

Background

When stimulating a patient with poor ovarian response for IVF, the maximal dose of gonadotropins injected is often determined by arbitrary standards rather than a measured response. The purpose of this study was to determine if serum FSH concentration during an IVF stimulation cycle reflects follicular utilization of FSH and whether serum FSH values may inform dose adjustments of exogenous FSH.

Methods

In this retrospective cross sectional study we studied 155 consecutive IVF cycles stimulated only with recombinant human FSH. We only included long GnRH agonist protocols in which endogenous FSH levels were suppressed. We correlated the serum concentration of cycle day (CD) 7 FSH with the number of oocytes retrieved, cleaving embryos and pregnancy rate.

Results

We found that a CD7 FSH concentration above 22 IU/L was associated with poor response regardless of the daily dose of FSH injected and a lower pregnancy rate.

Conclusions

We concluded that CD7 FSH concentration during stimulation could be used to guide FSH dosing in poor responders. If the CD7 FSH concentration is above 22 IU/L increasing the dose of FSH in an attempt to recruit more growing follicles is unlikely to be successful.

8. Klein NA, Battaglia DE, Fujimoto VY, Davis GS, Bremner WJ, Soules MR. Reproductive aging: accelerated ovarian follicular development associated with a monotropic follicle-stimulating hormone rise in normal older women. J Clin Endocrinol Metab 1996;81:1038–45.

Women experience a decline in fertility that precedes the menopause by several years. Previous studies have demonstrated a monotropic rise in FSH associated with reproductive aging: however, the mechanism of this rise and its role in the aging process are poorly understood. The purpose of this study was to characterize ovarian follicular development and ovarian hormone secretion in older reproductive age women. Sixteen women, aged 40-45 yr, with regular ovulatory cycles were studied. The control group consisted of 12 ovulatory women, aged 20-25 yr. Serum obtained by daily blood sampling was analyzed for FSH, LH, estradiol (E), progesterone, and inhibin (Monash polyclonal assay). Follicle growth and ovulation were documented by transvaginal ultrasound. Older women had significantly higher levels of FSH throughout the menstrual cycle. E, progesterone, LH, and inhibin levels did not differ between the two age groups when compared relative to the day of the LH surge. Ultrasound revealed normal growth, size, and collapse of a dominant follicle in all subjects. Older women had significantly shorter follicular phase length associated with an early acute rise in follicular phase E, reflecting accelerated development of a dominant follicle. We conclude that older reproductive age women have accelerated development of a dominant follicle in the presence of the monotropic FSH rise. This is manifested as a shortened follicular phase and elevated follicular phase E. The fact that ovarian steroid and inhibin secretion were similar to those in the younger women suggests that elevated FSH in women of advanced reproductive age may represent a primary neuroendocrine change associated with reproductive aging

9. Abdalla H, Nicopoullos JD. The effect of duration of coasting and estradiol drop on the outcome of assisted reproduction: 13 years of experience in 1,068 coasted cycles to prevent ovarian hyperstimulation. Fertil Steril 2010;94:1757–63.

OBJECTIVE:

To determine the effect of duration of coasting (Cd), estradiol levels at trigger (E(2)), and level of estradiol drop (E(2)d) on live birth rate (LBR) in cycle outcome.

DESIGN:

Retrospective analysis.

SETTING:

Hospital-based fertility clinic.

PATIENT(S):

A total of 1,068 coasted cycles (5.7% of total) of IVF/ICSI from 1996 to 2008.

INTERVENTION(S):

Coasting in IVF/ICSI cycles.

MAIN OUTCOME MEASURE(S):

Live birth rate and secondary cycle outcomes.

RESULT(S):

Mean Cd, E(2), and E(2)d were 4.7 days, 11,567 pmol/L, and 9,760 pmol/L, respectively. Maternal age, duration of subfertility, and serum FSH were significantly lower, and AMH (39.7 vs. 15.1 pmol/L) and prevalence of polycystic ovary syndrome (31.8% vs. 17.8%) significantly higher, in coasted cycles. Fertilization rate, clinical pregnancy rate, and LBR per cycle and implantation rate of 64.4%, 40.7%, 35.7%, and 24.7%, respectively, were demonstrated, with no significant difference in LBR in cycles coasted for up to 8 days or when divided according to E(2) or E(2)d. Lack of predictive capability on LBR was confirmed by receiver operator curve analysis which demonstrated areas under the curve of 0.51, 0.53, and 0.54 for E(2), Cd, and E(2)d, respectively.

CONCLUSION(S):

Although cycle numbers beyond 6 days are limited, coasting for up to 8 days does not affect LBR, and E(2) and E(2)d levels do not significantly affect cycle outcome.

10. Sher G, Zouves C, Feinman M, Maassarani G. ''Prolonged coasting'': an effective method for preventing severe ovarian hyperstimulation síndrome in patients undergoing in-vitro fertilization. Hum Reprod 1995;10:3107–9.

Over a 4 year period ending 1 January 1995, 51 women scheduled for in-vitro fertilization (IVF) and embryo transfer were inadvertently severely overstimulated with menotrophins, as evidenced by the development of > 29 ovarian follicles in association with peak plasma oestradiol concentrations of > 6000 pg/ml. Accordingly, these women were at great risk of developing life-endangering complications associated with severe ovarian hyperstimulation syndrome (OHSS). Treatment involved withholding the administration of both menotrophins and human chorionic gonadotrophin for a number of days, while continuing gonadotrophin-releasing hormone agonist until the plasma oestradiol concentration fell to < 3000 pg/ml ('prolonged coasting'). The mean number of oocytes retrieved was 21.0, while the mean number of embryos transferred per procedure was 5.4. There were 21 clinical pregnancies (i.e. pregnancy rate of 41% per oocyte retrieval), 19 of which resulted in live births (i.e. a live birth rate of 37% per oocyte retrieval). Two pregnancies miscarried and there were four multiple gestations (three sets of twins and one set of triplets). None of the women developed severe OHSS. Prolonged coasting is an effective method of preventing the occurrence of severe OHSS without necessitating the cancellation of the IVF cycle or compromising success rates.

11. Fluker MR, Hooper WM, Yuzpe AA. Withholding gonadotropins (''coasting'') to minimize the risk of ovarian hyperstimulation during superovulation and in vitro fertilization–embryo transfer cycles. Fertil Steril 1999;71:294–301.

Objective: To evaluate superovulation (SOV) and IVF-ET cycles in which E2 levels were allowed to decrease to restrain rapid follicular growth and minimize the risk of ovarian hyperstimulation syndrome.

Design: Retrospective series.

Setting: Tertiary care infertility practice.

Patient(s): Women who underwent SOV (n = 51) and IVF-ET (n = 93) treatment and who were at risk for OHSS.

Intervention(s): In SOV cycles, hMG was withheld (coasting) for >3 days before hCG administration, until follicular maturity was attained (≥3 follicles of ≥18 mm) and E2 levels decreased. In IVF-ET cycles, either follicular maturity was attained before coasting (n = 63), allowing hCG administration after E2 levels decreased by >25%, or coasting occurred before follicular maturation (n = 30), necessitating the administration of additional hMG after coasting.

Main Outcome Measure(s): Estradiol concentrations, follicle size, and pregnancy rates.

Result(s): Estradiol concentrations usually rose for ≥1 day after coasting began, then fell by ≥25% while follicle numbers and mean diameters increased. No spontaneous LH surges occurred, although four SOV cycles were canceled because of excessive follicular development. Of the women who received hCG, 11 of 47 (23% per cycle) conceived during SOV and 35 of 93 (37.6% per cycle) conceived during IVF-ET. Severe ovarian hyperstimulation syndrome developed in 1 woman who underwent IVF-ET.

Conclusion(s): Coasting can safely rescue overstimulated SOV and IVF-ET cycles characterized by an excessive rise in E2 levels and/or numerous incompletely mature follicles.

12. Levinsohn-Tavor O, Friedler S, Schachter M, Raziel A, Strassburger D,Ron-El R. Coasting—what is the best formula? Hum Reprod 2003;18:937–40.

Coasting is a method to decrease the incidence of ovarian hyperstimulation syndrome (OHSS), which involves withdrawing exogenous gonadotrophins until the serum estradiol (E2) level decreases. The application of this strategy, as it appears in the literature, has been variable, with heterogeneous criteria for initiating and ending the coasting process and as a result, reports of efficacy are inconsistent. In attempt to establish a recommended protocol for coasting we reviewed and analysed 10 relevant studies, found by a Medline search. Based on the data collected, coasting should be initiated when the serum E2 concentration exceeds 3000 pg/ml, but not unless the leading follicles reach a diameter

of 15–18 mm. Its duration should be limited to <4 days, thus, preventing the decrease in implantation and pregnancy rates that occur after longer periods of coasting. Administration of hCG should be withheld until serum E2 falls below 3000 pg/ml. Based on the published data, these suggested guidelines result in an acceptably low incidence of severe OHSS (<2%) and provide satisfactory fertilization and pregnancy rates (55–71% and 36.5–63% respectively). A multicentre randomized prospective study would help to confirm the effectiveness of this approach.

13. Moreno L, Diaz I, Pacheco A, Zuniga A, Requena A, Garcia-Velasco JA. Extended coasting duration exerts a negative impact on IVF cycle outcome due to premature luteinization. Reprod Biomed Online 2004;9:500–4.

Coasting, or withholding gonadotrophin administration while maintaining gonadotrophin-releasing hormone analogue until oestradiol drops to a safe concentration, is an alternative approach to prevent ovarian hyperstimulation syndrome (OHSS) in high responder patients. However, the length of this procedure has not been precisely studied. This paper is a retrospective study of 132 patients who showed a high response (oestradiol >4500 pg/ml and/or more than 20 follicles >17 mm) to ovarian stimulation and were coasted due to their high risk of developing OHSS, and evaluated the impact of the duration of coasting on IVF cycle outcome. Additionally, serum LH and progesterone concentrations were studied to investigate whether premature luteinization was present in these cycles and whether it might be related to coasting duration. A significant decrease in implantation rate was found when coasting was required for more than 4 days, together with a trend towards a higher cancellation rate. Premature luteinization was significantly elevated in women undergoing coasting compared with control women (34 versus 15.6%, P < 0.05). In the majority of patients who showed premature luteinization, coasting lasted ≥3 days. To conclude, prolonged coasting may affect the endometrium, anticipating the implantation window. These data may explain why some women undergoing extended coasting show a lower implantation rate compared with controls.

14. le Contonnec JY, Porchet HC, Beltrami V, Khan A, Toon S, Rowland M. Clinical pharmacology of recombinant human follicle-stimulating hormone. II. Single doses and steady state pharmacokinetics. Fertil Steril 1994;61:679–86.

OBJECTIVE: To assess the single-dose pharmacokinetics of a recombinant human FSH preparation (Gonal-F; Laboratoires Serono, Aubonne, Switzerland), administered by i.v., IM, and SC routes and its pharmacokinetics at steady state after multiple dosing by the SC route.

DESIGN: Twelve healthy down-regulated female volunteers received in random order three single doses of recombinant human FSH (150 IU, i.v., IM, and SC), with each administration separated by 1 week. The volunteers then received multiple recombinant human FSH doses by the SC route (150 IU one time per day) for 7 days. Follicle-stimulating hormone concentrations were measured by an immunoradiometric assay and an in vitro granulosa cell aromatase bioassay.

RESULTS: After a single administration, the pharmacokinetics of recombinant human FSH were well-described by a two-compartment model after i.v. administration and by a one-compartment model with first order absorption after IM or SC administration. The mean total clearance of FSH was approximately 0.6 L/h, and renal clearance accounted for one tenth of the total elimination after i.v. administration. The distribution half-life was close to 2 hours. The terminal half-life was nearly 1 day when estimated either by modeling the i.v. data set or from analysis of the terminal phase of the steady state pharmacokinetic curve or from the time taken to reach steady state after repeated SC administrations. After single IM and SC injection, two thirds of the administered dose was available systemically. The cumulation factor for repeated SC administration was approximately 3 when steady state was reached. The in vitro bioassay data confirmed these estimations. The temporal evolution of the bioassay:immunoassay ratio suggests either metabolic selection or activation of recombinant human FSH toward forms with greater in vitro bioactivity.

CONCLUSION: The estimation of the elimination half-life of approximately 1 day indicates that the maximal effect of a given dose of recombinant human FSH administered daily cannot be observed until 3 to 4 days of repeated administration. This indicates that, on a pure pharmacokinetic basis, physicians should wait at least 4 days to assess the efficacy of a given dose of recombinant human FSH and that they should not modify dosage too frequently.

15. Farhi J, Fisch B, Sapir O, Pinkas H, Ben-Haroush A. Effect of coasting on IVF cycle characteristics and outcome in short vs. long GnRH agonist protocols. Gynecol Endocrinol 2010;26:187–92.

Aims. To compare the results of IVF cycles following coasting in patients treated with long versus short GnRH agonist protocols.

Methods. A retrospective comparative study in which all women aged 35 years or less attending the IVF unit from 2000 to 2006 in whom coasting was used in GnRH agonist protocols were included. Data on coasting-related variables and outcome were collected from the files and compared between the short GnRH agonist (n = 78) and long GnRH agonist (n = 181) cycles.

Results. The short GnRH agonist cycles were characterized by higher E2 levels during coasting and longer duration of coasting than the long GnRH agonist cycles. Although the number of retrieved oocytes was lower following coasting in the short protocol, there was no difference between the groups in fertilization rate, number of high-quality embryos available for transfer, and pregnancy rate. Pregnancy rate in both protocols was negatively correlated to E2 level at initiation of coasting. The overall moderate and severe OHSS rate after coasting was 5.1% in the short-protocol group and 6.0% in the long-protocol group (p = 0.76).

Conclusions.The ovarian response curve to coasting is longer in the short than in the long GnRH-agonist protocol, but there is no significant difference in pregnancy or OHSS rates

16. Kovacs P, Matyas S, Kaali SG. Effect of coasting on cycle outcome during in vitro fertilization/intracytoplasmic sperm injection cycles in hyperresponders. Fertil Steril 2006;85:913–7.

Objective: To investigate the effect of coasting on IVF outcome in GnRH agonist cycles.

Design: Retrospective analysis.

Setting: Private IVF center.

Patient(s): Infertile couples undergoing IVF/intracytoplasmic sperm injection (ICSI) treatment (normal responders [control], hyper-responders [coasting] groups).

Intervention(s): Coasting to reduce the risk of ovarian hyperstimulation syndrome (OHSS) among hyper-responders.

Main Outcome Measure(s): Stimulation, embryology parameters, and pregnancy rate (PR).

Result(s): The average length of coasting was 2.2 days. Age and baseline FSH were comparable to control cycles. There were more follicles and oocytes in the coasting group, but the number of fertilized oocytes and embryos transferred were similar. Implantation rate (22.4% vs. 13.9%) was higher in the control group but the PRs were comparable (45.1% vs. 38.5%). Within the coasting group, baseline, stimulation, and embryology parameters were comparable between successful and unsuccessful cycles. Pregnancy rates were comparable after 1, 2, and 3 or more days of coasting (36.3% vs. 38.4% vs. 40%). Pregnancy rates were also comparable (28.5% vs. 35.7% vs. 44.4%) when groups were compared based on change in E2 (<25%, 25%–50%, >50%).

Conclusion(s): Coasting for 3 days can be used successfully in the management of the hyper-responding patients during IVF

17. Dhont M, van der Straeten F, de Sutter P. Prevention of severe ovarian hyperstimulation by coasting. Fertil Steril 1998;70:847–50.

Objective: To evaluate the efficiency of withholding gonadotropins and deferring the administration of hCG until E2 levels start dropping (coasting) in the prevention of ovarian hyperstimulation syndrome (OHSS) in a high-risk population.

Design: Retrospective case-control study.

Setting: In vitro fertilization program at a university center.

Patient(s): The case group consisted of 120 women undergoing ovarian stimulation for IVF who were considered to be at risk for ovarian hyperstimulation (serum E2 levels >2,500 pg/mL or >20 follicles at the time of hCG administration).

Intervention(s): Gonadotropin administration was withheld when serum E2 levels exceeded 2,500 pg/mL, and hCG administration was delayed until E2 levels dropped below 2,500 pg/mL. Outcomes were compared with those from 120 matched patients in whom serum E2 levels and the number of follicles at the time of hCG administration were comparable to those at the beginning of coasting (control group).

Main Outcome Measure(s): Incidence of moderate and severe OHSS. The number of oocytes retrieved and pregnancy rate (PR) were compared in both groups.

Result(s): Coasting decreased the incidence of moderate and severe OHSS. The odds ratio of severe OHSS in the coasting group was 0.11 (95% confidence interval 0.01–0.86). Although the number of oocytes was significantly lower in the coasting group (19.7 ± 0.6 versus 22.1 ± 0.6), coasting did not affect the PR (37.5% versus 36.7%).

Conclusion(s): Our study indicates that coasting is an efficient method for reducing the incidence and severity of OHSS without compromising the PR.

18. Moon HS, Joo BS, Moon SE, Lee SK, Kim KS, Koo JS. Short coasting of 1 or 2 days by withholding both gonadotropins and gonadotropin-releasing hormone agonist prevents ovarian hyperstimulation syndrome without compromising the outcome. Fertil Steril 2008;90:2172–8.

Objective: To evaluate the effect of short coasting, by withdrawing both gonadotropins and gonadotropin-releasing hormone (GnRH) agonist, on the prevention of severe ovarian hyperstimulation syndrome (OHSS) without compromising pregnancy outcome.

Design: Retrospective study.

Setting: Large urban medical center.

Patient(s): Forty-four women who had been coasted during controlled ovarian hyperstimulation (COH) for in vitro fertilization (IVF).

Intervention(s): When ≥20 follicles >15 mm with serum estradiol (E2) level of 4000 pg/mL were detected, both gonadotropins and GnRH agonist were withheld for 1 to 2 days.

Main Outcome Measure(s): Changes of serum E2 levels, number of oocytes retrieved, pregnancy rate.

Result(s): The mean serum E2 level fell from 7915 pg/mL at the onset of coasting to 3908 pg/mL on the day of human chorionic gonadotropin (hCG) administration. The mean number of oocytes retrieved and fertilization rate were 17.2% and 75.0%, respectively. Eighteen patients became pregnant (43.9%), and the implantation rate was 12.7%. Twenty-eight patients were coasted for 1 day, and 13 were coasted for 2 days. The mean decrease rate of serum E2 level was 45.3% in 1-day coasting and 26.4% (first day) and 75.3% (second day) in 2-day coasting. The pregnancy outcome was similar between both groups. After coasting, three mild and two severe cases of OHSS occurred.

Conclusion(s): Coasting for 1 or 2 days can be used successfully to prevent OHSS without compromising IVF cycle outcome.

19. Abdallah R, Kligman I, Davis O, Rosenwaks Z. Withholding gonadotropins until human chorionic gonadotropin administration. Semin Reprod Med 2010;28:486–92.

Withholding gonadotropins in women who exhibit high estradiol responses before follicles reach full maturation is called "coasting." Coasting, or suspending gonadotropin administration, can be an effective strategy for decreasing the risk of ovarian hyperstimulation syndrome (OHSS) while reducing cancelation rates. In in vitro fertilization cycles, mechanistically it is believed that withholding gonadotropins starves smaller follicles, induces apoptosis, and decreases

the potential for these follicles to elaborate vascular endothelial growth factor, a known mediator of OHSS. It is generally accepted that coasting should be initiated when the estradiol (E2) level is >3000 pg/mL in the setting of immature follicles. The human chorionic gonadotropin (hCG) trigger should be administered when the E2 level subsequently drops to a "safe" level. Cycle cancellation should be considered if, after 3 to 4 days of coasting, the E2 level remains excessively elevated. Oocyte retrieval may also be cancelled if the E2 level on the day after hCG trigger drops precipitously. In gonadotropin-releasing hormone agonist (GnRHa)-based protocols, one can consider withholding GnRHa administration if the E2 level continues to increase after a few days of coasting. Current data seem to show that the coasting period is short and/or is less likely to be required in GnRH-antagonist protocols as compared with GnRHa-based protocols. Large randomized control trials are still needed to establish the relative efficacy of coasting versus embryo cryopreservation in the context of OHSS prevention.

20. Knecht M, Ranta T, Feng P, Shinohara O, Catt KJ. Gonadotropin-releasing hormone as a modulator of ovarian function. J Steroid Biochem 1985;23:771–8.

GnRH and its agonist analogs exert direct inhibitory and stimulatory effects on the ovaries of animals from several species. In the immature follicle, GnRH inhibits the actions of FSH on an integrated array of biochemical responses that lead to follicular development and corpus luteum formation. GnRH also suppresses gonadotropin action in mature follicles, and stimulates certain ovarian processes such as steroidogenesis and oocyte maturation. The inhibitory and stimulatory effects of GnRH are mediated through the binding of the peptide to high-affinity receptors in granulosa and thecal cells. Recent studies have shown that GnRH action in the ovary is dependent upon calcium mobilization and probably operates through stimulation of phospholipid turnover and activation of protein kinase C.

21. Wadia PR, Mahale SD, Nandedkar TD. Effect of the human folliclestimulating hormone–binding inhibitor and its N-terminal fragment on follicle-stimulating hormone–induced progesterone secretion by granulosa cells in vitro. J Biosci 2007;32:1185–94.

Intrafollicular factors play an important role in folliculogenesis. The follicle-stimulating hormone (FSH)-binding inhibitor (FSHBI), purified by our laboratory from human ovarian follicular fluid, has been shown to suppress ovulation and induce follicular atresia/apoptosis in mice as well as impair fertility in marmosets, the new world monkeys. The octapeptide, a peptide corresponding to the N-terminal region of human FSHBI (hFSHBI), has been synthesized and also shows FSHBI activity in vitro. In the present study, we have attempted to identify the mechanism of action of the peptide in granulosa cell cultures. Rat granulosa cell cultures were treated with varying concentrations of the octapeptide or partially purified hFSHBI (gel chromatography fraction hGF2) in the presence or absence of human FSH (hFSH) and the amount of progesterone (P4) secreted in the culture supernatants after 3 h/48 h was estimated. Both hGF2 and the octapeptide failed to alter basal levels as well as 8-bromo cAMP-induced P4 production, while FSH-induced P4 secretion was inhibited in a dose-dependent manner. These studies reveal that the octapeptide, a fragment of FSHBI, and the native protein have similar activity in vitro and both compounds alter FSH action at the receptor level upstream of cAMP formation.

22. Weil SJ, Vendola K, Zhou J, Adesanya OO, Wang J, Okafor J, et al. Androgen receptor gene expression in the primate ovary: cellular localization, regulation, and functional correlations. J Clin Endocrinol Metab 1998; 83:2479–85.

Excess androgens are associated with a characteristic polyfollicular ovarian morphology; however, it is not known to what extent this problem is due to direct androgen action on follicular development vs. interference with gonadotropin release at the level of the pituitary or hypothalamus. To elucidate potential androgen effects on the ovary, we investigated the cellular localization of androgen receptor (AR) messenger ribonucleic acid (mRNA) in rhesus monkey using in situ hybridization. To investigate the regulation of ovarian AR gene expression, we compared the relative abundance of AR transcripts in monkeys during follicular and luteal phases of the menstrual cycle and in monkeys treated with

testosterone. To assess potential functional consequences of AR expression in the primate ovary, we compared AR mRNA levels with indexes of follicular cell proliferation and apoptosis in serial sections from individual follicles.

AR mRNA expression was most abundant in granulosa cells of healthy preantral and antral follicles in the primate ovary. Theca interna and stromal cells also expressed AR mRNA, but to a lesser degree than granulosa cells. No significant cycle stage effects were noted in AR mRNA levels; however, larger numbers of animals would be necessary to definitively establish a cycle stage effect. AR mRNA level was significantly increased in granulosa cells and was decreased in theca interna and stromal cells of testosterone-treated monkeys. Importantly, granulosa cell AR mRNA abundance was positively correlated with expression of the proliferation-specific antigen Ki-67 ($r = 0.91$; $P < 0.001$) and negatively correlated with granulosa cell apoptosis ($r = -0.64$; $P < 0.001$).

In summary, these data show that primate ovary AR gene expression is most abundant in granulosa cells of healthy growing follicles, where its expression is up-regulated by testosterone. The positive correlation between granulosa AR gene expression and cell proliferation and negative correlation with programmed cell death suggests that androgens stimulate early primate follicle development.

23. Slade P, Emery J, Lieberman BA. A prospective, longitudinal study of emotions and relationships in in-vitro fertilization treatment. Hum Reprod 1997;12:183–90.

Emotional and relationship assessments were completed by 144 couples at intake for in-vitro fertilization (IVF) and 6 months after either the identification of pregnancy or the discontinuation of treatment following three unsuccessful cycles. Women also completed emotional assessments at the time of pre-oocyte recovery and post-embryo replacement within each treatment cycle. At intake, women were more anxious than their partners and comparative norms, and were less positive than men about their marital and sexual relationships. Initial emotional assessments were not related to subsequent pregnancy, but at follow-up those who were pregnant were less depressed and more positive about their relationships. Within treatment cycles scores for women were higher after embryo replacement and the failure of pregnancy. First and last treatment cycles were associated with greater anxiety. High levels of confusion and bewilderment found during the initial cycle may indicate the need for better pretreatment information. Services must recognize the presence of high anxiety at intake and provide psychological care for those identified as particularly distressed. Emotional difficulties after failure of IVF treatment can be considered to be iatrogenic effects, and psychological services should be provided to minimize any negative psychological consequences of treatment.

24. Verhaak CM, Smeenk JM, van Minnen A, Kremer JA, Kraaimaat FW. A longitudinal, prospective study on emotional adjustment before, during and after consecutive fertility treatment cycles. Hum Reprod 2005;20:2253–60.

Background: A longitudinal study into the course of the emotional response to IVF from pre-treatment to 6 months post-treatment and factors that contributed to that course. Methods: A total of 148 IVF patients and 71 partners completed self-report questionnaires on anxiety, depression, personality characteristics, meaning of fertility problems, coping, marital relationship and social support at pre-treatment. Assessments of anxiety and depression were repeated immediately following the final treatment cycle and again 6 months later (follow-up). Results: Women showed an increase of both anxiety and depression after unsuccessful treatment and a decrease after successful treatment. Men showed no change in anxiety and depression either after successful or after unsuccessful treatment. In the 6 months after unsuccessful treatment, women showed no recovery. At follow-up, >20% of the women showed subclinical forms of anxiety and/or depression. Personality characteristics, meaning of the fertility problems, and social support determined the course of the emotional response. Conclusions: Most women adjusted well to unsuccessful treatment, but at follow-up, a considerable proportion still showed substantial emotional problems. Personality characteristics, pre-treatment meaning of the fertility problems and social support have demonstrated the adjustment to unsuccessful IVF in women. This allows early identification of women at risk as well as tailored interventions. © The Author 2005. Published by Oxford University Press on behalf of the European Society of Human Reproduction and Embryology. All rights reserved.

25. García-Velasco JA, Zuniga A, Pacheco A, Gómez R, Simon C, Remohi J, et al. Coasting acts through downregulation of VEGF gene expression and protein secretion. Hum Reprod 2004;19:1530–8.

BACKGROUND: This study was conducted to investigate the mechanisms by which coasting may be effective in decreasing the incidence of ovarian hyperstimulation syndrome (OHSS). METHODS: A total of 160 women (patients and oocyte donors) undergoing coasting and 116 controls were included in the study. Serum, follicular fluid and granulosa cells were collected on the day of oocyte retrieval. Vascular endothelial growth factor (VEGF) concentrations were determined using an enzyme−linked immunosorbent assay (ELISA). Real−time PCR was performed to evaluate VEGF gene expression in granulosa cells. Cell death was studied by flow cytometry using annexin V–fluorescein isothiocyanate (FITC) and counterstaining by propidium iodide, and double staining with CD45 monoclonal antibody was performed to distinguish the contamination of apoptotic leukocytes. RESULTS: Follicular cells aspirated from coasted patients showed a ratio in favour of apoptosis, especially in smaller follicles (48 versus 26%, P < 0.05). Follicular fluid determinations confirmed that coasting reduces VEGF protein secretion (1413 versus 3538 pg/ml, P < 0.001) and gene expression (2−fold decrease) in granulosa cells. Follicular fluid VEGF protein levels positively correlated with follicular size (r = 0.594, P = 0.001) and estradiol production (r = 0.558, P = 0.038). Women who underwent coasting showed a comparable IVF cycle outcome; however, a higher cancellation rate was found in cycles that were coasted. CONCLUSIONS: Coasting affects all follicles through apoptosis, especially immature follicles, without affecting oocyte/endometrial quality. The significant decrease found in VEGF expression and secretion explains why coasting is clinically effective in reducing the incidence and severity of OHSS.

COMENTARIOS DEL EDITOR

A pesar de las limitaciones muestrales y del carácter retrospectivo de este trabajo, resulta al menos interesante comprobar que, frente a los múltiples protocolos empíricos ineficaces en la pobre respondedora para los que "más" estímulo gondadotrópico es mejor sin ninguna base fisiopatológica que lo justifique, estos autores desarrollen una metodología con fundamento fisiopatológico que relaciona la saturación de los receptores de granulosa para la FSH y la posibilidad de incrementar la su eficacia mediante la adición de testosterona.

La apuesta de una hipótesis en la que discontinuar la estimulación gonadotrópica y administrar testosterona transdérmica, para que la maquinaria enzimática de la granulosa continúe en condiciones más eficaces, parece quedar respaldada por los resultados y abre una nueva puerta para el optimismo terapéutico en este complejo grupo de pacientes.

Autor para correspondencia: Dr. Vicente López Villaverde.

E-mail: vlopezvilla@gmail.com