ISSN 2185-6230 (Online)

Journal of REPRODUCTION ENGINEERING

July 2011
Vol. 14, No. 1: 1-12
J. Reprod. Engineer.

Japan Society for
Reproduction Engineering

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Journal of REPRODUCTION ENGINEERING JRE

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J. Reprod. Engineer.
2011; Volume 14, Number 1

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http://sre.ac.affrc.go.jp/Contents.htm

Journal of REPRODUCTION ENGINEERING JRE

= 症例報告 =

加齢女性生殖機能低下に対するdehydro-epiandrosterone併用による不妊治療
Combined therapy for reproductive dysfunction of aged female with dehydro-epiandrosterone

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要旨

ヒトにおける卵巣機能の加齢変化は個体差が極めて大きく、卵巣機能および予備能評価法が求められる。抗ミューラー管ホルモン(AMH)値は発育卵胞数と相関すると考えられ、卵巣予備能の指標として注目されている。筆者は卵巣機能を賦活目的としてdehydro-epiandrosterone (DHEA)を投与下に体外受精−胚移植(IVF-ET)などのassisted reproductive technologies (ART)を施行し、その成績と下垂体機能、卵巣機能、AMH値を比較した。Premature ovarian failure (POF)と診断された53症例には過排卵誘発施行前にDHEAを3ヶ月投与し、血清のFSH値(10 mIU/ml以下)かつtestosterone (T)値(20 ng/ml以上)になったことを確認した後にDHEA、recombinant FSH (r-FSH)併用療法を施行した。DHEA投与によりFSH値は49.5から28.2 IU/mlに、T値は14.7から62.6 ng/mlに変化した。FSH値が10 mIU/ml以下かつT値が20 ng/ml以上に改善し、血清E_2値上昇を認めた症例が43例(改善率83%)に達し、17症例(妊娠率32%)でIVF-ETによる妊娠が成立した。過排卵誘発を実施した157例(27–49歳)を年齢別に4群に分類し、血中AMHを測定した。AMH値は35歳未満では23.0 ± 17.5 pmol/lであったが、年齢とともに減少し、41歳以上では7.2 ± 5.3 pmol/lへと顕著に減少し、加齢変化を確認できた。採卵数は20 pmol/l未満の2群は20 pmol/l以上の群に比して有意に低下した。これまで卵胞成熟はゴナドトロピン、ステロイド等の血中濃度を指標として評価されてきた。ゴナドトロピンは卵巣血流による組織移行を介して卵胞成熟に関与する。hCG投与直後、採卵時の血中および卵胞液中のhCG濃度から組織移行率を観察した結果、hCG卵胞液内移行率は加齢にともない有意に低下し、同時に採卵数も減少した。その原因として排卵直前の血管新生低下が推察された。非分割卵を後方視的に解析すると、非分割卵の80%以上が精子不侵入で占められ、これが卵非分割の最大原因であることが示唆された。さらにその率は加齢とともに増加する傾向を認めた。成熟卵は第二減数分裂中期で静止しているが、観察した非分割卵には様々な異常が認められ、紡錘体形態異常と染色体不整列の両者を合わせもったものが最も高頻度であった。35歳以上の群で紡錘体系異常の発生率が増加したが、核系異常の頻度は年齢による変化は少ない傾向を認めた。

キーワード: 加齢, 卵巣, 卵, dehydro-epiandrosterone (DHEA), hCG, 組織移行, 非分割卵

序論

ヒト加齢に伴うゴナドトロピン感受性低下は卵巣機能低下をもたらす。そのため加齢婦人は過排卵誘発に抵抗性であることが多く、成熟卵を得られない症例をしばしば経験する。血清のFSH 10 mIU/ml以上、E_2 25 pg/ml未満であり、human menopausal gonadotropin (hMG)製剤による排卵誘発に抵抗性を示す症例は子宮内膜非薄化、成熟卵胞数減少および最大卵胞径の

投稿日: 2010年11月9日
掲載決定日: 2011年6月15日
ウェブサイト事前公開日: 2011年6月16日

増加遅延を認める場合が多く、卵の質的低下は受精率、妊娠率の低下を招く。その頻度は加齢に伴い増加し、治療は困難を極めることが多い。

一方、卵巣機能の加齢変化は個々の症例間で変動幅が極めて大きいこともよく知られており、卵巣機能およびその予備能を多面的に評価する方法が求められる。抗ミュラー管ホルモン(Anti-mullerian hormone: AMH)は発育卵胞、前胞状卵胞から分泌され、その血中濃度は発育卵胞数と相関すると考えられ[3]、加齢に従い卵巣機能が低下するとFSHは上昇し、発育卵胞数が減少するとAMHは低下する。AMHは、性周期の影響を受けにくいため、卵巣予備能の指標となると考えられている。

本論文では、卵巣機能を賦活させると考えられるdehydro-epiandrosterone (DHEA)[2]を投与した場合、下垂体機能、卵巣機能、AMH値で示される卵巣機能予備能などのパラメーターの変化や改善の有無、また体外受精-胚移植(IVF-ET)によるARTの成績について検討した。さらに非分割に終わった卵の状態を後方視的に観察した。

対象(材料)と方法

検討1. FSH高値であり排卵誘発に抵抗性を示す症例に対するdehydro-epiandrosterone (DHEA)併用した場合の不妊治療

Day 3（月経初日をDay 0とする)の血清FSH 10 mIU/ml以上、E_2 25 pg/ml未満である、hMG製剤による過排卵誘発に抵抗性であり、子宮内膜の非薄化、卵胞数の減少、最大卵胞径の増加遅延を認め、血清総testosterone (T) が20 ng/ml以下、の条件を満たす53症例（34.5 ± 3.6 歳）をDiminished Ovarian Reserve (DOR)と定義して対象とした。さらにこれらの不妊治療経過を検討した

検討2. hCG組織移行率を指標とした卵巣機能の評価

これまで卵胞成熟はゴナドトロピン、ステロイド等の血中濃度を指標として評価されてきた。ゴナドトロピンは卵巣血流による組織移行を介して卵胞成熟に深く関与し、hCG 投与により顕著に卵巣血流が増加することが報告されている[6]。hCG は採卵決定後に一定量をone shot静注し、一定時間後に採卵が行われる。本研究は外因性hCG 組織移行の薬動力学的解析が、卵巣機能評価に有用であるかを検討した。投与直後、採卵時の血中および卵胞液中のhCG 濃度は常法に従い測定した。体重の1/13を患者血液量と仮定し、採卵時の卵胞液中/血中hCG濃度×100を組織移行率と定義し、解析を行った。過排卵誘発を施行した66症例を対象とした。

検討3. AMHを指標とした卵巣予備能の評価

過排卵誘発を実施し、AMHを測定した症例(27–49歳)を年齢別に4群(34歳以下、35–37歳、38–40歳、41歳以上)に分類し、比較した。

検討4. IVFにおける非分割卵の後方視的観察

Conventional-IVFを試みたが非分割に終わった症例のうち、廃棄胚の観察に同意が得られた38症例（年齢25–44歳）から提供された134卵を対象とした。

POFと診断された症例には過排卵誘発施行前に性ステロイド前駆体であるDHEAを3ヶ月間投与(50 mg/日)した[4]。その後、血清FSH 10 mIU/ml、Tが20 ng/ml以上になったことを確認し、DHEA、r-FSHまたはhMG併用療法を施行し、最大卵胞径が18 mm以上に達するまで投与した。卵胞数、最大卵胞(主席卵胞)径の大きさ、子宮内膜厚、FSH、E_2およびT値を測定した。さらに、加齢とAMH値との関連、AMH値と内分泌所見の相関さらにART成績との関連を観察した。AMHはEIA AMH/MIS KIT (IMMUNOTECH, Marseille, France)を用いて測定した。

媒精2–3日後の非分割卵を2%パラホルムアルデヒド固定し、抗α-tubulin 抗体を用いて免疫染色し、propidium iodideを用いて核染色した。観察には共焦点レーザー顕微鏡(LSM510 META, Carl Zeiss Microimaging, Jena, Germany)を用いて核、紡錘体を撮影し、写真上で以下に示す基準に従って解析した。卵の異常は、紡錘体および核由来に大別した。さらに紡錘体は1) 形態異常(染色体は整列しているが、紡錘糸に乱れがある)、2) 多極紡錘体(樽の先端の部分に位置する紡錘体極は通常2ヶ所であるが、3ヶ所存在するもの、この場合は染色体も不整列）、3) Premature Chromosome Condensation（PCC: 卵由来の紡錘体の他に、精子由来の紡錘体様核が観察される)に細分類した。核は1) 染色体不整列(紡錘糸は樽の先端をすぼめたきれいな形状をしているが、染色体が整列していない)、2) 核断片化(紡錘糸は観察されず、DNAも染色体の形状をとらず、卵全体に散ってバラバラである)、3) 染色体凝集（紡錘糸は観察されず染色体が一ヶ所に凝集している)、4) 前核停止(DNAが前核様で停止しており、実体顕微鏡下では明瞭な前核が観察されない)に細分類した。精子については卵細胞質への精子侵入について観察した。

結果

検討1. FSH高値であり排卵誘発に抵抗性を示す症例に対するDHEA併用の不妊治療

高FSH、低E_2、低T値を認める症例にDHEAを併用投与した。DHEA投与前後における内分泌値を表1に、卵胞数、最大卵胞径、子宮内膜厚を表2に示す。症例全

表1. ヒト女性不妊治療患者に対するDHEA投与による血中ホルモン(FSH, E_2, T)値の変化

	投与前	投与後
FSH (mIU/ml)	49.5 ± 11.8[a]	28.2 ± 2.4[b]
E_2 (pg/ml)	58.1 ± 19.8[a]	71.6 ± 43.0[b]
T (ng/ml)	14.7 ± 12.5[a]	62.6 ± 43.5[b]

症例数=53。値は、平均 ± 標準偏差。[a,,b]同行異符号間に有意差あり(P < 0.05)。

表2. ヒト女性不妊治療患者に対するDHEA投与による卵胞数、最大卵胞径および子宮内膜厚の変化

	投与前	投与後
卵胞数	1.6 ± 0.97[a]	3.4 ± 1.2[b]
最大卵胞径(mm)	12.6 ± 1.90[a]	18.8 ± 1.68[b]
子宮内膜厚(mm)	6.8 ± 1.3[a]	10.2 ± 1.3[b]

症例数=53。値は、平均 ± 標準偏差。[a,,b]同行異符号間に有意差あり(P < 0.05)。

表3. ヒト女性年齢別の採卵時におけるhCG投与前後の残存率および卵胞内移行率と採卵数

年齢	症例数	体重 (kg)	血中hCG (mIU/ml)		残存率 (%)[#]	卵胞液中hCG (mIU/ml)	hCG卵胞内移行率 (%)	採卵数(個)
			hCG投与時	採卵時				
34以下	16	49.2 ± 6.6	2686.4 ± 344.5	241.2 ± 62.8	9.1 ± 2.4	172.3 ± 59.7	72.4 ± 18.7[a]	5.6 ± 3.7[a]
35–37	21	54.1±8.0	2446.6 ± 313.8	172.2 ± 89.4	6.9 ± 3.0	125.1 ± 76.3	71.0 ± 19.9[a]	3.6 ± 2.6[b]
38–40	12	53.3 ± 10.2	2513.2 ± 448.9	177.0 ± 80.4	7.1 ± 3.0	116.7 ± 75.8	60.0 ± 24.2[ab]	2.2 ± 2.3[b]
41以上	17	53.6±7.8	2475.0 ± 365.4	214.0 ± 108.9	8.6 ± 3.9	120.8 ± 88.5	53.5 ± 20.3[b]	1.9 ± 2.8[b]

値は、平均 ± 標準偏差。[#]採卵時の血中hCG/hCG投与時の血中hCG量×100。[a,b]同行列異符号間で有意差あり(P < 0.05)。

表4. ヒト女性加齢にともなう抗ミュラー管ホルモン(AMH)およびFSH値

年齢	症例数	AMH (pmol/l)	FSH (mIU/ml)
34以下	20	23.0 ± 17.5[a]	10.2 ± 5.7
35–37	15	20.3 ±13.6[ab]	9.9 ± 3.4
38–40	23	13.1 ±11.9[bc]	18.2 ± 28.5
41以上	18	7.2 ± 5.3[c]	26.0 ± 29.6

症例数=76。値は、平均 ± 標準偏差。[a–c]同列異符号間で有意差あり(P < 0.05)。

体におけるFSH、T平均値の変化は49.5から28.2 IU/ml、14.7から62.6 ng/mlであった。FSHが10 mIU/ml以下かつTが20 ng/ml以上に改善された症例が43例に達し、その改善率は83%であった。hMG反応性が改善されて卵胞数、最大卵胞径、子宮内膜厚が有意に増加した。53症例中17症例にIVF-ETによる妊娠が成立した。その妊娠率は32%であった。DHEA補充療法がhMG感受性を改善し、卵胞発育ひいては卵の質向上に関与する可能性が示唆された。

検討2. hCG組織移行率を指標とした卵巣機能の評価

表3に示すように、投与されたhCGの血中濃度は採卵までに減少し、その残存率は年齢別群間で有意な差を認めなかった。一方、卵胞液中に移行したhCGの濃度を血中濃度で除したhCG卵胞液内移行率は34歳以下群から年齢が上昇するにつれ減少し、37歳以下の2群に比して41歳以上群で有意に低下(P < 0.05)した。同様に採卵数も減少し、34歳以下群に比してそれ以上の3群は有意な低下(P < 0.05)を認めた。一方、取得した卵の受精率、分割率に年齢は影響しなかった。

検討3. AMHを指標とした卵巣予備能の評価

AMH値は34歳以下では23.0 ± 17.5 pmol/lであったが、年齢とともに減少し、41歳以上では7.2 ± 5.3 pmol/lへと顕著に減少し、加齢変化を確認できた(表4)。表5にまとめたようにIVF施行例をAMH値20以上、10以上20未満、0以上10未満(単位: pmol/l)の3群に分類すると、0–10 pmol/ml群の年齢は他の2群に比して有意に高かった。採卵率は10 pmol/l未満の群において、有意に低かった。また採卵数は20 pmol/l未満の2群は20 pmol/l以上の群に比して有意に低下した。一方、比較した3群間でAMH値は得られた卵の受精率、分割率等に影響しなかった。AMH値は、DHEA投与前後で変化しなかった。

検討4. IVFにおける非分割卵の後方視的観察

女性患者の年齢分布を34歳以下、35–37歳、38歳以上の3群に分けて比較した。まず卵細胞質内への精子侵入を観察すると全ての群で非分割卵の80%以上が精子不侵入で占められ、これが卵非分割の最大原因であることが示唆された(図1)。さらにその率は、加齢とともに増加する傾向を認めた。

ヒト成熟卵は第二減数分裂中期で静止しているが、IVF後の非分割卵には様々な異常が認められ、紡錘体形態異常(図2)と染色体不整列の両者を組み合わせたものが最も高頻度であった。また、35歳以上の群で紡錘体系異常の発生率が増加したが、核の異常の頻度においては年齢による変化は少ない傾向を認めた。

表5. ヒト女性年齢別の採卵時における抗ミュラー管ホルモン(AMH)値および採卵率・体外受精成績

AMH (pmol/l)	年齢	採卵率#	採卵数	受精率 (%)	分割率 (%)
20以上	35.5 ± 2.6[a]	87% (32/37)	3.8 ± 3.7[a]	64.0 ± 36.8	56.8 ± 39.4
10以上20未満	36.0 ± 4.2[a]	83% (30/36)	2.1 ± 2.4[b]	65.1 ± 42.1	64.8 ± 42.4
0以上10未満	38.3 ± 5.2[b]	55% (46/84)	1.2 ± 2.0[b]	55.1 ± 47.3	54.7 ± 47.0

値は、平均 ± 標準偏差。#1個以上の卵が採取できたIVF周期/全IVF周期。[a,b]同列異符号間で有意差あり(P < 0.05)。

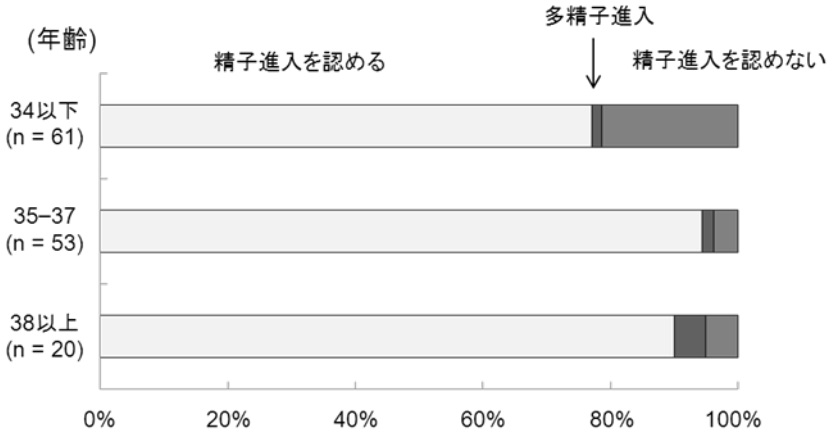

図1. ヒト女性年齢別の採卵・体外受精における非分割卵の精子侵入率

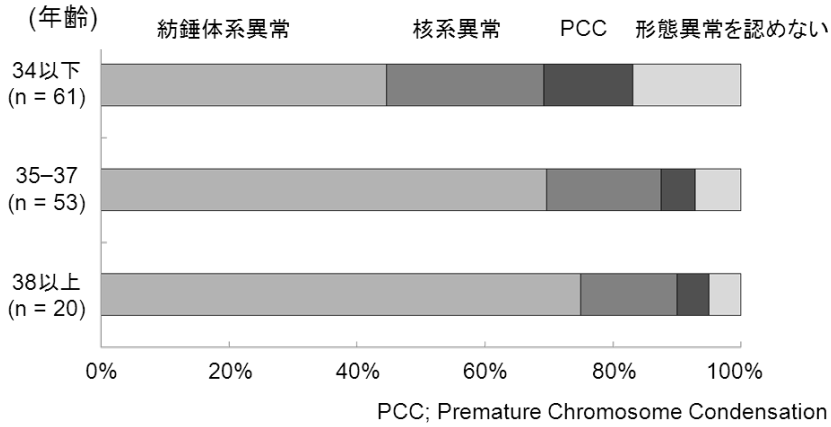

図2. ヒト女性年齢別の採卵・体外受精における非分割卵の核の状態

考察

DHEAは副腎皮質でコレステロールから生成されるステロイドホルモンであり、性腺組織で容易にテストステロンおよびエストロゲンに変換される。DHEAは成年初期に産生量がピークに達し、加齢に伴い産生量が減少するホルモンとして知られている。卵巣機能不全の発症にDHEAがどのように関与するのか、また、卵巣機能不全にDHEA補充療法がどのように作用しているかは、不明な点が多い。Barad & Gleicherは高年齢者に対する排卵誘発にDHEAを併用投与し、卵胞発育促進を認め、妊娠例が得られたと報告した[1]。そしてDHEA補充療法の作用機序は、卵巣のhMG感受性を改善し、卵胞発育ひいては卵の質向上に寄与したと考察している。

AMHは発育卵胞、前胞状卵胞から分泌され、その血中濃度は発育卵胞数と相関すると考えられている。加齢に伴い卵巣機能が低下するとFSHは上昇し、発育卵胞数が減少することによりAMHは低下する。AMHは性周期の影響を受けにくいため、卵巣予備能の良い指標となると考えられている[5]。

本論文ではPOFと診断された症例にDHEAを先行投与し、83%にホルモン値の改善を認め、これらの症例に引き続いて過排卵誘発を施行した結果、妊娠例を得た。これらの結果は、上述したBarad & Gleicher [1]の考察を支持する。

また、本論文では卵巣機能の指標としてAMH値を観察し、表4に示すように加齢に伴う低下を認めた。さらに表5にまとめたように、AMH値は採卵率、取得卵数に影響することを認めた。これらの結果は、性周期により値が大きく変動するFSHに比して安定的な卵巣機能評価に寄与する可能性が考えられ、今後さらに症例を集積

してAMH値における限界低値の設定が求められる。一方、AMH値は取得卵の受精率、分割率には影響しなかった。

hCGのenzyme immunoassayは内因性の下垂体性ホルモンと免疫学的交叉性が極めて低く(hCG:100、LH:0.7、FSH:0.06)、ほぼhCGの特異的測定が可能である。One shot静注されたhCGはタンパク質性ホルモンの体内動態追跡の指標として適している。本論文で示された結果は、ホルモン血中濃度と標的細胞が存在する卵胞液中に濃度は必ずしも比例せず、加齢とともにゴナドトロピンの卵胞内移行率が低下している可能性が示唆され、その原因として排卵直前の血管新生低下が推察されるが、今後組織学的観察を含めた詳細な検討が求められる。

本論文で述べた、1. DHEAによる血中ホルモン値の正常化、2. AMHを指標とする卵巣予備能の評価、3. hCGの血中動態および卵胞液中への移行率測定は、多面的解析により加齢に伴う卵巣機能低下を把握する試みである。研究4.は結果として未分割に終わった卵に関する後方視的解析を行うことにより、研究1.、2.、3.で解析した卵巣がどのような卵を形成したかに関する情報を提供する。卵が未分割に終わったことは、まず精子が侵入していたか否か、精子侵入卵において未分割の原因が精子側にあるのか、卵側にあるのか、両者であるか詳細に解析することにより、形成された卵の質的評価、さらには次回治療方針決定に重要な情報を提供する。図2に示すように精子未侵入が80%を占めたことは、同時に精子機能に関する多面的な解析が不可欠であることも示唆している。当院を受診する患者の年齢層は高く、38歳以上が約60%を占めている。一般に加齢婦人の不妊治療は躊躇されがちである。当院が行ってきた試みは、多面的な評価による卵巣機能および卵質の把握、およびそれらの情報に基づいた適切な卵巣機能賦活を行うことにより、加齢かつDOR症例においても妊娠の可能性があることが示された。

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Japan Society for Reproduction Engineering

J. Reprod. Engineer. 2011; 14: 7–12.
http://sre.ac.affrc.go.jp/Contents.htm

Journal of REPRODUCTION ENGINEERING JRE

= Original Article =

Ovulatory effects of an extract from Maitake mushroom in patients with polycystic ovary syndrome

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ABSTRACT

This study aimed to determine the effects of an extract from Maitake mushroom ("SX-fraction", SX) on ovulation in patients with polycystic ovary syndrome (PCOS). Thirty patients were randomly assigned to either of the following two treatment arms for three months: the SX group (n = 15), which prescribed 207 mg/day of SX, and the control group (n = 15), which received 7.5 g/day of Shakuyaku-Kanzo-To (SKT; a Chinese herbal medicine). Ovulation was judged by the change of basal body temperature and ultrasonography. During the treatment period, 66.7% of the patients in the SX group ovulated compared with 30.8 % in the SKT group; however, the difference was not statistically significant (p = 0.0581). During the same period, ovulation was confirmed in 22 out of 45 menstrual cycles (48.9%) in the SX group, while for the SKT group ovulation was confirmed in 15.4% (P = 0.0011). Estradiol and the estradiol/testosterone ratio also showed a statistically significant increase (P = 0.032 and 0.038, respectively) when those compared before and after treatment with SX. The SX group was 14 times more likely than the SKT group to induce ovulation (95% CI: 1.093 - 196.45). These results summarized; 1) SX was effective in the treatment of PCOS and 2) The SX regimen improved the ovulation rate independent of insulin resistance. In conclusion, SX appears to promote aromatization of testosterone to estradiol in granulosa cells.

Key words: polycystic ovary syndrome (PCOS), treatment, Maitake mushroom extract (SX-fraction), insulin resistance, aromatization

INTRODUCTION

Polycystic ovary syndrome (PCOS), characterized clinically by oligomenorrhea or anovulation, hyperandrogen, and polycystic ovaries, is one of the most common endocrinopathies and a major cause of infertility, affecting approximately 4.6% (3.5%-11.2%) of women of reproductive age [12]. Although there is an internationally accepted definition of PCOS [19], ethnic differences in the disease should be considered. Therefore, in 2007, the Japan Society of Obstetrics and Gynecology proposed slightly different criteria for Japanese women [10].

In the treatment of PCOS, clomiphene citrate (CC) has been used for many years as the standard medication to induce ovulation [17]. According to the step-by-step clinical protocol, if CC treatment fails (unsuccessful ovulation or pregnancy), patients are recommended for consecutive gonadotropins, ovarian surgery, and in vitro fertilization. Such treatments, however, are expensive and associated with some risk of side effects.

Submitted: January 3, 2011
Accepted: June 10, 2011
Advance Publication in Website: June 11, 2011

On the other hand, it has been found that hyperinsulinemia might contribute to hyperandrogenism, representing metabolic and hormonal aspects of PCOS. Therefore, drugs to improve hyperinsulinemia and insulin resistance (e.g., metformin) have been introduced for the treatment of PCOS [2, 26]. These drugs, however, have been reported to be associated with several adverse effects, as in the case of metformin, which is widely known to induce lactic acidosis, a rare side effect [3, 4]. It is then desirable to develop or explore certain medications with less adverse reactions.

Maitake is an edible mushroom belonging to the family Polyporaceae in the order Aphyllophorales. Maitake has been shown to be capable of lowering blood glucose levels [9, 13–15, 18, 21] and an extract responsible for that activity has been identified and named "SX-fraction". It is a glycoprotein with an average molecular weight of around 20,000 Daltons, and has been reported to improve insulin resistance and associated clinical symptoms, including PCOS. In the present study, we examined SX-fraction, an extract from Maitake (*Grifola frondosa*) mushroom, as a potential alternative therapeutic modality for PCOS. SX-fraction proved to be effective in ovulation induction in our pilot study, which was presented at the 22nd annual meeting of Traditional Medicines in Tokyo, 2005 [24].

MATERIALS AND METHODS

Materials

In the present study, we used the following plant source products: 1) SX-fraction (Maitake Products, Inc. NJ, U.S.A.) and 2) Shakuyaku-Kanzo-To (SKT), a commonly prescribed Japanese traditional herbal medicine (Tsumura & Co., Tokyo, JAPAN), with less adverse reaction, as used for the treatment of PCOS [20].

Study design and subjects

An unblinded prospective randomized controlled 3-month trial was designed and conducted at Loma Linda Clinic from March to December 2007. This study included 30 Japanese women (21-37 years old) with newly diagnosed PCOS. Diagnosis of PCOS was based on the 2007 Japan Society of Obstetrics and Gynecology criteria, as follows: 1) menstrual abnormality, 2) polycystic ovary and 3) hyperandrogenism and/or elevated basal luteinizing hormone (LH) and normal basal follicle stimulating hormone (FSH). As for LH and FSH, both LH $\geq$ 7 mIU/ml and LH/FSH $\geq$ 1 are required; in the case of obesity (body mass index, BMI $\geq$ 25), however, LH/FSH $\geq$ 1 is acceptable to diagnose PCOS [10]. Trans-vaginal ultrasound was performed by the same examiner to diagnose PCOS morphologically, i.e., 1) the number of follicles is more than 10, and 2) the sizes of follicles are less than 9 mm.

The patients who met the medical criteria were randomly allocated to either of the two treatment arms. Allocation was made by opening sealed envelopes that contained random assignment cards to either the SX-fraction group or the SKT group. The investigators and the patients were not blinded to the treatment arms, due to a difficulty in preparing indistinguishable placebos for SX-fraction and SKT. The patients were instructed to take SX or SKT three times before meals per day.

The basal body temperature (BBT) records of the subjects were examined and anovulations lasting for more than three months were confirmed. Patients with known history of hypogonadotropic hypogonadism, diabetes, or liver, renal, or heart disease were excluded from the study. Anovulatory patients were given medroxyprogesterone acetate (MPA) to induce a withdrawal bleeding, and a blood test was run to detect hormonal and metabolic disorders as a routine procedure. The respective heights, weights and BMIs of all patients were also recorded at entry into the study.

The study was approved by the Institutional Review Board at Inui Institute for Frontier Reproductive Medicine and Infertility, and informed consent was obtained from all participants prior to the study.

Medications and monitoring

The patients were assigned to two groups: the SX-fraction group (n = 15), which received 207 mg/day of SX-fraction, and the SKT group (n = 15), which received 7.5 g/day of SKT. The status of the patients' menstruation and ovulation was monitored by recording menstrual bleeding and BBT every morning. BBT has been confirmed as a relatively accurate method for retrospective identification of ovulation [16]. In addition, trans-vaginal ultrasonography was used to observe follicle development (a follicle size of $\geq$ 12 mm was considered a developing follicle) or ovulation. The participants were instructed to maintain any routine exercises and/or follow the dietary regimen they have been on before joining this study. During the study two patients from the SKT group dropped out upon personal

Table 1. Characteristics of 28 women with the polycystic ovary syndrome before and after treatment with SX-fraction and SKT

	SX-fraction (n=15)			SKT (control) (n=13)		
	At baseline	After treatment	P	At baseline	After treatment	P
Age	31.53 ± 5.11	–	–	28.1 ± 4.42	–	–
BMI	23.01 ± 7.04	–	–	22.6 ± 5.18	–	–
LH	13.61 ± 4.36	11.41 ± 5.19	0.079	12.90 ± 4.87	11.90 ± 5.22	0.525
LH/FSH	2.43 ± 0.66	2.34 ± 0.96	0.614	2.51 ± 1.68	2.28 ± 1.03	0.619
T	56.95 ± 27.64	54.55 ± 30.59	0.517	35.71 ± 7.90	36.14 ± 10.79	0.917
E_2	39.72 ± 12.03	83.38 ± 71.37	0.032	37.17 ± 11.38	51.24 ± 34.95	0.209
PRL	10.95 ± 5.84	13.38 ± 4.35	0.252	10.31 ± 5.45	12.47 ± 4.61	0.124
E_2/T	0.87 ± 0.50	1.83 ± 1.62	0.038	1.09 ± 0.36	1.80 ± 1.78	0.186
FBS	82.47 ± 8.26	82.53 ± 7.10	0.970	82.42 ± 6.68	82.08 ± 6.84	0.852
IRI	5.61 ± 4.26	6.36 ± 4.73	0.193	7.18 ± 3.41	6.57 ± 6.03	0.548
HOMA-IR	1.21 ± 0.99	1.30 ± 0.98	0.603	1.47 ± 0.72	1.40 ± 1.40	0.781

Mean ± SD are presented.
SX-fraction; extract from Maitake mushroom, SKT; Shakuyaku-Kanzo-To, BMI; body mass index, T; testosterone, E_2; estradiol, PRL; prolactin, FBS; fasting blood sugar, IRI; fasting insulin, HOMA-IR: homeostasis model assessment for insulin resistance.

Table 2. Prognostic factors for ovulation

	Odds ratio	95% CI
Age	1.00	0.798–1.255
Treatment	14.66	1.093–196.452
LH	0.85	0.642–1.136
T	0.97	0.931–1.018
BMI $\geq$ 25	0.73	0.036–15.108
HOMA-IR $\geq$ 2	5.83	0.251–135.641

Treatment; SX-fraction (compared to SKT), T; testosterone,
CI: confidence Interval, HOMA-IR: homeostatic model assessment index,
BMI:body: mass index.

requests unrelated to a safety concern.

Assays

Blood was drawn in the morning, between day 3 and day 5 of the withdrawal bleeding or menstruation periods, and the samples were sent to the same laboratory for biochemical analysis. IRI (fasting insulin), FBS (fasting blood glucose), HOMA-IR (homeostatic model assessment index), E_2 (estradiol), LH, FSH, LH/FSH (LH to FSH ratio), T (testosterone), E_2/T (estradiol to testosterone ratio), and PRL (prolactin) levels were measured at baseline and after three months. A quantitative determination of the serum hormones was performed by chemiliuminescense immunoassay (CLIA).

Statistical Analyses

The continuous variables are expressed as means ± SD (standard deviation). Fisher's exact test was used to analyze the statistical difference between the treatment arms in "ovulation rate" (percentage of ovulated patients) and "ovulation cycle rate" (percentage of menstrual cycles with ovulation). Paired t-test (two-tailed) was used to assess the statistical difference between the values at baseline and those after the treatment in hormonal and metabolic parameters. Multiple logistic regression analysis was used to examine the effects of treatments after adjusting the effects of other factors. Statistical significance was accepted when p-value is less than 0.05. Any data on the two discontinued patients were excluded from the analysis. Data analysis was then performed using Statistics Package for Social Sciences (SPSS) Version 11.5 for Windows (IBM Japan, Tokyo, Japan).

RESULTS

Ten of the 15 patients in the SX-fraction group ovulated (ovulation rate = 66.7 %) during the treatment period compared with 4 of 13 patients in the SKT group (ovulation rate = 30.8 %), but the difference was not statistically significant (P = 0.0581) (Figure 1). On the other hand, ovulation was confirmed in 22 out of a total 45 cycles (ovulation cycle rate = 48.9%) in the SX-fraction group during that period. This rate was significantly higher (P = 0.0011) than that of the SKT group, which was only 6 out of a total 39 cycles (ovulation cycle rate = 15.4%) (Figure 2). These results show that SX-fraction was more effective than SKT in

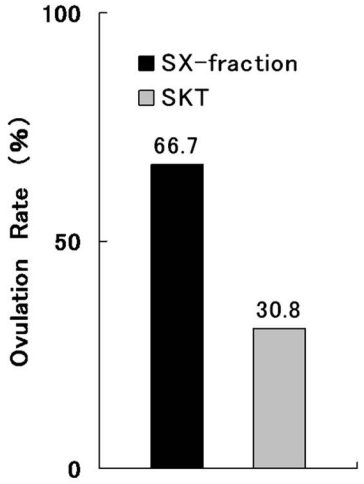

Figure 1. Ovulation rates in extract from Maitake mushroom (SX fraction) and Shakuyaku-Kanzo-To (SKT) groups
Data were calculated based on the number of patients with polycystic ovary syndrome who ovulated during the administrateion of SX fraction and SKT. The rates were not different significantly (P = 0.0581).

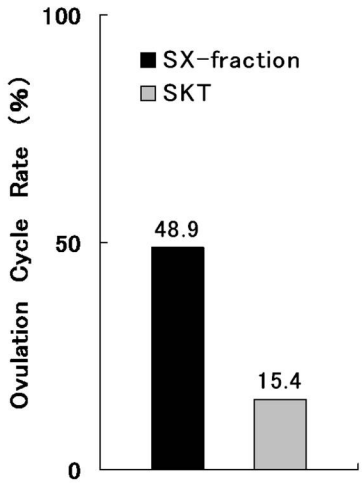

Figure 2. Ovulation cycle rates in extract from Maitake mushroom (SX fraction) and Shakuyaku-Kanzo-To (SKT) groups
Ovulation was confirmed 48.9% of total cycle in SX fraction and 15.4% in SKT. The rates were different significantly (P = 0.0011).

inducing ovulation in patients with PCOS.

Table 1 shows various parameters of the subjects (n = 28) before and after the treatment with SX-fraction and SKT (control). There were no statistically significant differences at baseline between the study groups. In the treatment group with SX-fraction, LH decreased from 13.61 ± 4.36 to 11.41 ± 5.19, although it was not statistically significant (P = 0.079). E_2 and the E_2/T ratio (a higher E_2/T ratio represents a greater aromatization activity) were significantly increased after administration of SX-fraction (P = 0.032 and 0.038, respectively). The results of multiple logistic regression analysis (Table 2) show that SX-fraction was almost 14 times more likely than SKT to induce ovulation (odds ratio of 14.66 at 95% confidence interval for 1.093–196.45).

DISCUSSION

Maitake is well known to have potent immune stimulatory and anti-tumor activities, which have been shown to be superior to those in other mushrooms. Moreover, such super- bioactivities of Maitake are known to stem from an active ingredient called "D-fraction" [8]. Besides this fraction, another bioactive compound has been recently isolated from Maitake and been so called "SX-fraction". It is a water-soluble glycoprotein with an average molecular weight of 20,000 Daltons. Recent reports have shown that SX-fraction is capable of modulating blood glucose levels [9, 13–15, 18, 21]. Other studies on SX-fraction have indicated that it might help reduce blood pressure [1, 11, 22, 23] and serum lipids [6, 11]. Thus, it is conceivable that SX-fraction may be a useful natural product to prevent the development of insulin resistance syndrome or metabolic syndrome. Furthermore, it may be used as an alternative agent for the treatment of PCOS.

Based on the findings of the current reproductive endocrinology, insulin resistance and excessive production of androgen from ovary are more likely to impede ovulation. Probably they could be the major contributors to the pathogenesis of PCOS. For example, insulin sensitizers such as metformin have been shown to improve the hormonal imbalances associated with PCOS, resulting in an improvement of ovulatory function. Nonetheless, some patients may experience gastrointestinal symptoms during metformin therapy, and in rare cases, metformin may cause fatal lactic acidosis. Accordingly, we investigated the ovulatory and endocrinological, metabolic effects of SX-fraction as a possible safer alternative treatment for PCOS. The results of multiple logistic regression analysis, adjusting the effects of confounding variables at baseline that might have affected the treatment outcomes, showed a remarkable treatment effect of SX-fraction (Table 2).

As far as hormonal and metabolic changes in the patients are concerned, E_2 and the E_2/T ratio were

significantly increased after administration of SX-fraction (Table 1). These results indicate that SX-fraction may increase the aromatization in the granulosa cells, which promotes the biosynthesis of E_2 from T, thereby resulting in the increased serum E_2/T ratio. Although SX-fraction has been reported to likely improve insulin resistance, no significant changes were observed in FBS, IRI, or HOMA-IR by SX-fraction in our PCOS patients. This discrepancy could be due, at least in part, to the limited sample size of patients with insulin resistance or other factors. The complete picture of the pharmacological effects of SX-fraction on PCOS remains to be elucidated; however, to a certain extent, SX-fraction may yet share some similarities with SKT. Takahashi and Kitao have reported a significant increase of the E_2/T ratio in PCOS patients who received SKT. This implies that SKT may increase the activity of aromatase, promoting the synthesis of E_2 from T and subsequently lowering the serum testosterone levels [20]. Fulghesu et al. reported that increased aromatization activity stimulated by exogenous gonadotropins is a reflection of hyperinsulinemic status in patients with PCOS [7]. We assumed that this may partially explain the pharmacological effects of SX or SKT on PCOS.

In addition, locally produced androgens and estrogens are important in the hormonal regulation of follicular development: increased E_2 and decreased T productions would facilitate the increase in follicle size. The decrease in E_2 production observed during atresia of the dominant follicle is believed to be the direct result of decreased aromatase activity in granulosa cells [25]. Our findings also suggest that SX-fraction appears to stimulate the conversion of T to E_2 (aromatization) and/or to reverse the inhibited aromatization of testosterone in granulosa cells, which demonstrates an antagonistic effect on follicular development.

Furthermore, it is known that race and ethnicity would influence the phenotypic manifestation of PCOS; for instance, Japanese women have been shown to express less clinical and biochemical hyperandrogenism. Therefore, it is of interest to investigate the effects of SX-fraction on PCOS with respect to racial and ethnical differences in the future.

Regarding the safety of SX-fraction, it would be reasonable to assume that the risk would be minimal for the following reasons. Firstly, SX-fraction is derived from an edible mushroom with a history of human experience for more than a thousand years. Secondly, SX-fraction showed no toxicity in single-dose and repeated-dose toxicity studies (unpublished in-house data; Maitake Products, Inc. NJ, U.S.A). Moreover, there have been no reports suggesting its possible toxicity in the laboratory and clinical studies with SX-fraction conducted so far [18, 21–23], and no serious adverse events have been reported since it was launched in the United States in 2004 (unpublished in-house data; Maitake Products, Inc. NJ, U.S.A.). SX-fraction was actually well tolerated without any problem in our present study.

A few limitations in the present study should be noted. Since the sample size of this trial was relatively small, it may reflect the wide range of the confidence interval of odds ratio in the multiple logistic regression analysis. Additionally, due to the small sample size of insulin-resistant subjects, i.e., 5 of 28 (17.8 %) of HOMA -IR$\geqq$ 2.0, we could not show a statistically significant effect of SX-fraction on the improvement of insulin resistance. However, the endocrinological mechanism of SX-fraction would be explained by increased aromatization after treatment. Further studies to explore the relationship between E_2, T, E_2/T, and insulin resistance are expected. Then, a large-scale study should be required to overcome such a limitation in the study (More recently, Chen et al. conducted a large scale study and confirmed the effect of SX-fraction to induce ovulation in PCOS patient) [5]. Furthermore, this study was not blinded, so that it is nearly impossible to rule out the involvement of some inevitable bias associated with investigators and/or patients.

In conclusion, SX-fraction was effective in anovulatory patients with PCOS, demonstrating the significant increases in E_2 and the E_2/T ratio. Multiple logistic regression analysis also indicated that SX-fraction regimen indeed improved the ovulation rate after adjusting for the effect of other factors.

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Journal of REPRODUCTION ENGINEERING　生殖工学会誌
2011年7月発行
発行者: 日本生殖工学会 (SRE)
代表: 柏崎 直巳
発行所: 日本生殖工学会 (SRE)
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