Jaffe RC, Ferguson-Gottschall SD, Gao W, Beam C, Fazleabas AT. Histone deacetylase inhibition and progesterone act synergistically to stimulate baboon glycodelin gene expression. *J Mol Endocrinol* 2007;**38**:401–407.

Kämäräinen M, Seppälä M, Virtanen I, Andersson LC. Expression of glycodelin in MCF-7 breast cancer cells induces differentiation into organized acinar epithelium. *Lab Invest* 1997;**77**:565–573.

Klentzeris LD, Bulmer JN, Seppälä M, Li TC, Warren MA, Cooke ID. Placental protein 14 in cycles with normal and retarded endometrial differentiation. *Hum Reprod* 1994;**9**:394–398.

Koistinen H, Seppälä M, Nagy B, Tapper J, Knuutila S, Koistinen R. Glycodelin reduces carcinoma-associated gene expression in endometrial adenocarcinoma cells. *Am J Obstet Gynecol* 2005;**193**:1955–1960.

Mandelin E, Lassus H, Seppälä M, Leminen A, Gustafsson JA, Cheng G, Butzow R, Koistinen R. Glycodelin in ovarian serous carcinoma: association with differentiation and survival. *Cancer Res* 2003;**63**:6258–6264.

Nishida M. The Ishikawa cells from birth to the present. *Hum Cell* 2002; **15**:104–117.

Oehninger S, Coddington C, Hodgen G, Seppälä M. Factors affecting fertilization: endometrial placental protein 14 reduces the capacity of human spermatozoa to bind to the human zona pellucida. *Fertil Steril* 1995;**63**:377–383.

Okamoto N, Uchida A, Takakura K, Kariya Y, Kanzaki H, Riittinen L, Koistinen R, Seppälä M, Mori T. Suppression by human placental protein 14 of natural killer cell activity. *Am J Reprod Immunol* 1991;**26**:137–142.

Owen GI, Richer JK, Tung L, Takimoto G, Horwitz KB. Progesterone regulates transcription of the p21(WAF1) cyclin- dependent kinase inhibitor gene through Sp1 and CBP/p300. *J Biol Chem* 1998;**273**:10696–10701.

Seppälä M, Wahlstrom T, Julkunen M, Vartiainen E, Huhtala L. Endometrial proteins as indicators of endometrial function. In: Tomoda Y, Mizutani S, Narita O, Klopper A (eds). *Placental and Endometrial Proteins: Basic and Clinical Aspects*. Utrecht, The Netherlands: VNU Science Press, 1988, 35–42.

Seppälä M, Taylor RN, Koistinen H, Koistinen R, Milgrom E. Glycodelin: a major lipocalin protein of the reproductive axis with diverse actions in cell recognition and differentiation. *Endocr Rev* 2002;**23**:401–430.

Shabani N, Mylonas I, Kunert-Keil C, Briese V, Janni W, Gerber B, Friese K, Jeschke U. Expression of glycodelin in human breast cancer: immunohistochemical analysis in mammary carcinoma in situ, invasive carcinomas and their lymph node metastases. *Anticancer Res* 2005;**25**:1761–1764.

Shapiro GI. Cyclin-dependent kinase pathways as targets for cancer treatment. *J Clin Oncol* 2006;**24**:1770–1783.

Shiozawa T, Nikaido T, Nakayama K, Lu X, Fujii S. Involvement of cyclin-dependent kinase inhibitor p27Kip1 in growth inhibition of endometrium in the secretory phase and of hyperplastic endometrium treated with progesterone. *Mol Hum Reprod* 1998;**4**:899–905.

Smid-Koopman E, Blok LJ, Kuhne LC, Burger CW, Helmerhorst TJ, Brinkmann AO, Huikeshoven FJ. Distinct functional differences of human progesterone receptors A and B on gene expression and growth regulation in two endometrial carcinoma cell lines. *J Soc Gynecol Investig* 2003; **10**:49–57.

Taylor RN, Lundeen SG, Giudice LC. Emerging role of genomics in endometriosis research. *Fertil Steril* 2002;**78**:694–698.

Telimaa S, Kauppila A, Ronnberg L, Suikkari AM, Seppälä M. Elevated serum levels of endometrial secretory protein PP14 in patients with advanced endometriosis. Suppression by treatment with danazol and high-dose medroxyprogesterone acetate. *Am J Obstet Gynecol* 1989;**161**:866–871.

Toki T, Mori A, Shimizu M, Nikaido T, Fujii S. Localization of apoptotic cells within the human endometrium and correlation between apoptosis and p21 expression. *Mol Hum Reprod* 1998;**4**:1157–1164.

Tung L, Mohamed MK, Hoeffler JP, Takimoto GS, Horwitz KB. Antagonist-occupied human progesterone B-receptors activate transcription without binding to progesterone response elements and are dominantly inhibited by A-receptors. *Mol Endocrinol* 1993;**7**:1256–1265.

Uchida H, Maruyama T, Nagashima T, Asada H, Yoshimura Y. Histone deacetylase inhibitors induce differentiation of human endometrial adenocarcinoma cells through up-regulation of glycodelin. *Endocrinology* 2005;**146**:5365–5373.

Uchida H, Maruyama T, Ono M, Ohta K, Kajitani T, Masuda H, Nagashima T, Arase T, Asada H, Yoshimura Y. Histone deacetylase inhibitors stimulate cell migration in human endometrial adenocarcinoma cells through up-regulation of glycodelin. *Endocrinology* 2007;**148**:896–902.

Waites GT, Wood PL, Walker RA, Bell SC. Immunohistological localization of human endometrial secretory protein, 'pregnancy-associated endometrial alpha 2-globulin' (alpha 2-PEG), during the menstrual cycle. *J Reprod Fertil* 1988;**82**:665–672.

Wood PL, Waites GT, MacVicar J, Davidson AC, Walker RA, Bell SC. Immunohistological localization of pregnancy-associated endometrial alpha 2-globulin (alpha 2-PEG) in endometrial adenocarcinoma and effect of medroxyprogesterone acetate. *Br J Obstet Gynaecol* 1988;**95**: 1292–1298.

Wu J, Xue L, Weng M, Sun Y, Zhang Z, Wang W, Tong T. Sp1 Is Essential for p16 expression in human diploid fibroblasts during senescence. *PLoS one* 2007;**2**:e164.

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特　集　子宮内膜症の診療

異所性子宮内膜症

Extrapelvic endometriosis, bladder endoemtriosis, and rectosigmoid colon endometriosis

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子宮内膜症の病巣部位は多岐にわたっている．一般的に発症頻度の高い，卵巣や腹膜以外に，直腸，膀胱，尿管，腹壁，横隔膜，肺などに発症することがある．ことに，骨盤内では消化管および尿路系への子宮内膜症の浸潤あるいは発生を認めることの頻度が比較的高く，また臨床症状である骨盤痛と月経困難症は高度であることが多い．それぞれの臓器の専門家と産婦人科と協力して治療にあたることが望ましい．

Key Words 直腸子宮内膜症，膀胱子宮内膜症，呼吸器子宮内膜症，異所性子宮内膜症

はじめに

子宮内膜症病巣は一般的に骨盤腔内，とくに子宮，卵巣，および腹膜に存在することが多い．しかし，骨盤内においても女性生殖器以外に浸潤する子宮内膜症や，骨盤腔以外に存在する子宮内膜症病変も認められる．きわめてまれな症例では，前立腺のホルモン治療後の男性の腹腔内にも子宮内膜症病巣が存在したという報告もある．

通常と異なる部位に子宮内膜症病巣が存在するものを異所性子宮内膜症と呼ぶことが多いが，明確な定義はなされていない．本稿では，異所性子宮内膜症を女性生殖器以外で発生する子宮内膜症と定義して使用することにする．異所性子宮内膜症の発症部位は（表1），解剖学的に骨盤内の病変と骨盤外病変にわけて考えると，臨床症状に関連して理解しやすい．骨盤内の異所性子宮内膜症とは，直腸・膀胱・尿管などに発症した子宮内膜症であり，また，骨盤外の異所性子宮内膜症には，回腸・空腸・臍部・胸膜・肺・中枢神経系などの発症がある．

以上のように，子宮内膜症の発症部位は多岐にわたり産婦人科医が遭遇する可能性の低い発症部位もあるが，他科からの依頼により対応が必要になることもあり，また子宮内膜症の発症要因の理解を深めることもできるため，異所性子宮内膜症の病態については理解を深めておく必要がある．

異所性子宮内膜症の発症部位

1．小骨盤腔内の異所性子宮内膜症

小骨盤腔は子宮内膜症が好発する部位であるため，いわゆる生殖器以外の部位にも子宮内膜症が発症し，発症頻度が低い異所性子宮内膜症のなかでは，比較的経験することがある疾患である．発

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表1　異所性子宮内膜症の発症部位と臨床症状

発症部位	月経に随伴する症状	慢性症状
ダグラス窩 (直腸・尿管)	月経困難症，性交時痛，排便時痛	慢性骨盤痛・性交時痛
消化管 (S状結腸，虫垂，空腸，回腸)	排便時痛，下血，腸閉塞	慢性骨盤痛・排便時痛
膀胱・尿管	血尿	尿管閉塞
横隔膜・肺	気胸	
表在性部位 (臍部，会陰切開部，腹部切開部)	疼痛，腫脹	硬結

症部位としては，消化管（直腸・S状結腸，虫垂，空腸，回腸），尿路（膀胱・尿管）が主たる部位である．いずれも偶発的に見つけられることが多いが，一方，症状を伴う場合は重症な症例が多く，外科・泌尿器科と連携したうえで加療を行う必要が生じる．また，これらの骨盤腔内の異所性子宮内膜症はダグラス窩子宮内膜症（いわゆる深部子宮内膜症）や子宮腺筋症などの重症例で合併することが多く，主訴である疼痛も強く，また加療後の再発率も高く治療に苦慮することが多い[1,2]．

小骨盤腔内の異所性子宮内膜症は，deep endometriosis（深部子宮内膜症）といって一括されて論じられていることが多い．深部子宮内膜症という名称を用いたこと，および直腸腔中隔からの子宮内膜症発症の可能性が指摘された経緯もあり病名の混乱があるが，本来はダグラス窩周囲子宮内膜症といった表現のほうが誤解を招きにくいと考えられる．直腸，S状結腸，膀胱，尿管などの子宮内膜症病巣は個別にあるわけではなく，病巣の広がりかたによって症状が生じる部位が異なるだけである．

深部子宮内膜症は，Donnezらの論文によって胎生期の遺残組織により発生が発症機序として有名ではあるが，臨床的にはこのような発症によるものはあったとしてもわずかであると考えられる．深部子宮内膜症は，子宮内膜症病巣によって生じた癒着によりダグラス窩が閉鎖し，そのため見かけ上，深部に内膜症があるように見えるものが大部分であり，胎生期の遺残組織により直腸腔中隔に単独で発症する子宮内膜症はきわめてまれであると考えている[3]．

直腸子宮内膜症のみならず，膀胱子宮内膜症においても同様の議論があり，また発症要因については混乱しているのが現状である．しかし膀胱においても，膀胱筋層のみから発症して，ほかに骨盤内に子宮内膜症がない症例がきわめてまれであることや，われわれの経験している症例においても，膀胱子宮内膜症の症例は，膀胱壁に隣接して腹膜子宮内膜症病変および子宮腺筋症があり，腹膜子宮内膜症あるいは子宮体部の子宮腺筋症が浸潤したと考えるほうが理解しやすい．

ダグラス窩・直腸・膀胱の子宮内膜症に関しては，胎生期組織遺残による発症もあるものの，骨盤子宮内膜症が派生して発症した症例が大多数と考えられる．

2．小骨盤腔外の異所性子宮内膜症

1）肺，横隔膜子宮内膜症

小骨盤腔以外では，肺，横隔膜に発症する異所性子宮内膜症の頻度が比較的高い．腹水の腹腔内での流動は，ダグラス窩→上行結腸およびモリソン窩→右横隔膜下といった移動があることから，月経血の逆流による発症が多いとされている[1]．腹腔鏡で手術施行の際に腹腔内を観察していると，腹腔内の洗浄液は頭低位をとった場合，左横隔膜下よりも右横隔膜下のほうに貯留した洗浄液の量が多いことに気がつく．このような腹水動態に沿った月経血の逆流が，横隔膜における異所性子

宮内膜症発症機序の一つであると推察されている．

その機序を示唆するように，横隔膜下の子宮内膜症も約60%は右のみであり，左側のみの発症は約5%との報告もある．しかし，横隔膜子宮内膜症の症例における骨盤内の子宮内膜症合併頻度に関しては，約20%という報告から約80%というものまであり[5,6]，報告者の専門が胸部外科あるいは産婦人科によって異なり，手術手技の違いもあることから（開胸手術と胸腔鏡，および開腹手術と腹腔鏡），報告者によるバイアスが含まれている可能性が高い．いずれにしても，横隔膜・肺の子宮内膜症は一定頻度で骨盤病変を伴っていることには注意が必要である．

2）臍部，腹壁，腹壁瘢痕，会陰切開部瘢痕に発症する子宮内膜症

腹壁瘢痕や会陰切開部に子宮内膜症が発症するとの報告もある．腹壁瘢痕への異所性子宮内膜症は妊娠中期の帝王切開が関連しているとされ，また会陰切開の瘢痕は分娩に伴った子宮内容掻爬との関連も可能性があり，子宮内膜症の発症原因の一つが子宮内膜の異所性接着にあるということの一つの根拠になっている．臍部や鼠径部（腹壁）の子宮内膜症の報告例も散見されるが，臍部子宮内膜症は骨盤病変を合併していることは少ないが，鼠径部子宮内膜症と骨盤病変との関連性については明確になっていない．

3）小腸，虫垂の子宮内膜症

憩室への子宮内膜症や虫垂炎として診断されたものが子宮内膜症である症例も散見されている．この場合，外科的治療は憩室炎や虫垂炎と同様であるが，骨盤子宮内膜症の合併がある場合，それに対する治療を総合的にどうするかなど，外科医と婦人科医での連携が必要である．

4）男性に認められる子宮内膜症

前立腺癌の治療後にホルモン剤（エストロゲン）の投与を長期的に受けた症例の urethral crest 近傍に，子宮内膜症が発症したという報告がある．男性の urethral crest 近傍はミューラー管の遺残があり，エストロゲン療法によって子宮内膜への化生が促進されたと考えられ，ミューラー管遺残組織による子宮内膜症発症の一つの根拠となると考えられる．

■■ 異所性子宮内膜症の診断

1．小骨盤腔内の異所性子宮内膜症の診断

いわゆる深部子宮内膜症であれば，月経困難症，性交時痛（dyspareunia），慢性骨盤痛，内診によるダグラス窩の圧痛が重要な所見である（表1）．通常の子宮内膜症と同様に，採血における CA125 などの腫瘍マーカーや MRI による骨盤内の検索も重要であり，とくに子宮腺筋症を合併している症例において，腺筋症病巣の広がりを評価するには MRI が最も有用である．また，消化器症状を伴うような症例では注腸検査により，結腸などの外方からの圧排，および強度のひきつれなどを検出しておくことも，加療方針を決めるうえで必要となる．

最近報告されている方法に，超音波ゼリーを膣内および直腸内に注入して MRI を施行する方法がある．われわれの症例においても，この方法により直腸内の癒着病変の描出が可能であった（図1）．直腸子宮内膜症病変の検出は術前には困難なことも多く，外科的治療に望む場合，月経困難症に対する治療に際しては術前の評価で直腸病変が困難な場合があり，術中に直腸の検査（内視鏡および超音波検査）が可能な体制にすることと，直腸の disc resection または segmental resection といった術式が術中にフレキシブルにとれるようにしておくことが必要な症例もある．

深部子宮内膜症患者においては病歴の聴取と理学的所見がきわめて重要である．内診と直腸診による圧痛点と，主訴による疼痛発生点が比較的一致することが診断の根拠となり，またこのように圧痛点が一致していることが外科的治療の有効性を示唆する．しかし一方，理学的診断（内診および直腸診）ではダグラス窩閉鎖症例の約50%しか検出することはできないことも注意が必要である．

疼痛を主訴とする子宮内膜症の発症を疑われる患者の診断においては，疼痛部位，疼痛発生の周

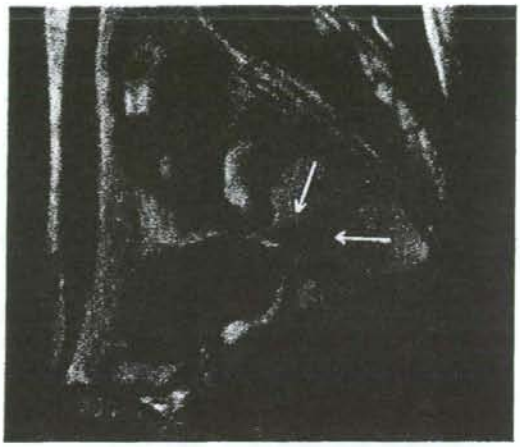


図1　S状結腸子宮内膜症のMRIによる診断
MRIゼリー法により検出された，S状結腸における子宮内膜症病変部位．

期性，消化器症状の有無などを検討しておくことが必要であり，ことに外科的治療の前にはより詳細な検討が必要である．

2．小骨盤腔外の異所性子宮内膜症の診断

臓器によって異なるが，それぞれの臓器における月経時の出血と腫張が特徴である．これに伴って肺・胸膜に病巣が存在すれば周期的に気胸を生じる．また，臍部に発症した子宮内膜症病巣であれば，月経時に一致した臍部からの出血と腫張である．いずれにしても好発臓器は比較的限定されるので，その部位によって発症する症状が月経周期に依存していることが診断のヒントとなり，最終診断は病理診断が必要である．

■■■ 異所性子宮内膜症の治療

異所性子宮内膜症の発症部位は多岐にわたるが，いずれの子宮内膜症病巣も基本的な治療は外科的切除となる．しかし，臨床症状や臓器の部位によって判断が異なってくる．薬剤による治療と経過観察のみで再発を認めなかったとの報告もあり，発症した部位と症状を考慮してから治療する必要がある．一般的な治療方法は通常の子宮内膜症の治療と同様であり，候補となる治療方法を表2に示した．

1．小骨盤腔内の子宮内膜症

1）消化管（S状結腸，直腸，虫垂）子宮内膜症の治療法

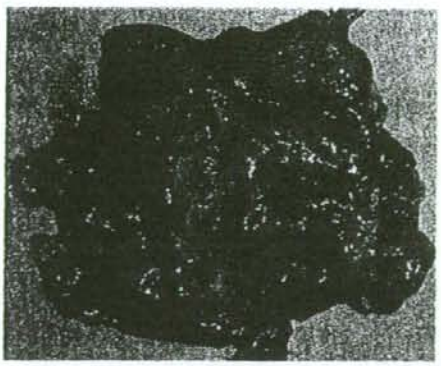
消化管の異所性子宮内膜症は，骨盤内の子宮内膜症に合併しやすい疾患である．排便時の痛み，月経時の下血，月経時の著明な消化器症状，性交時痛などの症状が強い場合は，直腸診や下部消化管検査（造影検査および内視鏡検査）などを施行して，消化管の全層切除が必要な症例かどうかを術前に十分評価しておく必要がある．

消化管に発見される異所性子宮内膜症には比較的症状が強い症例が多く，より根治的な治療により約80～90%は症状が改善すると報告されている．多数例の外科的治療による深部子宮内膜症症例の外科的治療（約80～90%は腹腔鏡下手術）による切除術の治療効果をみると，完全に病巣を切除をした症例でも数年後の再発率は約30～40%あり，とくに若年者においては再発率が高い傾向があることは注意が必要である[1)2)7)]．

症状が強い症例においては外科的切除が第一選択である．消化管の全層に病巣が及ぶ場合は全層切除が必要になり，また全層に及ばない場合は内膜症病巣の部位を切除し，その後，漿膜面を縫合するという手技がとられることもある．術者の技

表2　異所性子宮内膜症の治療法

	治療方法	利点	欠点
外科的治療法	胸腔鏡 腹腔鏡	・手術時に病巣部位の病理が確認できる. ・根治術を行えば，疼痛の治療効果が高い. ・再発率が薬剤による治療より少ない.	・外科的手術の合併症が生じうる. ・ダグラス窩の処置においては，直腸・結腸損傷の可能性があり，十分なインフォームドコンセントと同時に，準備が必要である.
内科的治療法	薬剤による治療法 (NSAIDs, GnRH, ピル，aromatase inhibitor など)	・手術などの侵襲はない. ・GnRH は術後投与において，手術による疼痛改善効果の延長が期待される. ・閉経が近い患者においては，薬剤治療の後に閉経になり，症状が緩和されると期待される.	・卵巣機能抑制による副作用が出現する. (GnRH の場合) ・排卵を抑制する治療であるため，妊娠を望む患者には適さない. ・治療による病巣の縮小はあるが消失はない. ・術前投与の有効性が認められている薬剤はない. ・薬物治療により外科的切除を困難にする場合もある.



○：子宮内膜症病巣

図2　S状結腸子宮内膜症
結腸・小腸などの消化管に発症する子宮内膜症病巣は多発性のものが約60%以上を占めると報告されている．この症例においても多発性の子宮内膜症病巣を認めた．

量と，施設における外科医との十分な連携をとったうえで術式を決定する必要がある．腹腔鏡下または，小切開を加えた腹腔鏡補助下の手術例の報告も増えてきていて，とくに欧米の施設では積極的に消化管の部分切除が行われている．われわれの施設においてもS状結腸，直腸，虫垂などに異所性子宮内膜症病変が存在していると考えられ，疼痛などの症状が重篤な症例の場合，十分な消化管前処置をしたうえで切除を行っている．図2にダグラス窩閉塞およびS状結腸に異所性子宮内膜症を伴った症例の腹腔鏡下手術における切除標本を示した．

消化管の子宮内膜症に関しては，ここに示すように多発性の病巣を示している症例が多く，切除による治療を行う場合は残存病巣をつくらないよう注意が必要である．消化器症状を伴った子宮内膜症患者で，消化管病変がある症例は切除によって劇的に症状が改善する可能性が高く，術前の評価が大切であるとともに，偶発的に消化管の内膜症病変が発見された場合にも術中に対応できる術前処置と，外科医との連携が必要とされる．また術後の治療に疼痛緩和目的で薬剤による治療

(GnRH，Danazol など）が追加される場合があり，妊孕性よりも疼痛治療を優先する場合にはGnRH製剤の術後投与が術後の再発率を下げる可能性が指摘されている．しかし，術前のGnRH投与は病巣部位の線維化が強くなり，内膜症病巣の切除が困難となることがあり注意が必要である．

根治的切除後（子宮全摘および卵巣切除後）にも残存した一部の卵巣や，子宮内膜症組織から子宮内膜症が進展する症例もまれにあり，根治術後のエストロゲン剤投与を行わざるをえない場合は細心の注意が必要である．難治性子宮内膜症病変の治療にaromatase inhibitorの使用も試みられていて，閉経後の骨盤腔内に子宮内膜症が進展した症例に有効であったとの報告もあり，aromatase inhibitorは今後，子宮内膜症の治療薬として一つの選択肢となる可能性がある．

2）膀胱・尿管子宮内膜症の治療法

膀胱・尿管のいずれにしても泌尿器科との連携が必要である．症状を伴う場合は外科的な治療が優先される．膀胱筋層に浸潤する子宮内膜症は比較的認められるが，尿管の筋層まで浸潤する子宮内膜症はきわめてまれであり，大部分の症例においては尿管周囲を剝離することにより，周囲の内膜症病巣は切除が可能である．しかし，尿管に子宮内膜症が浸潤している症例では，尿管膀胱新吻合や尿管尿管吻合が必要になる．

膀胱表面の子宮内膜症に関しては，骨盤の子宮内膜症の治療法に準じて切除が基本である．尿管口の部分に近い場合は処置が困難なこともあるが，膀胱底部などにある内膜症病変は腹腔鏡下に切除が可能である．尿管口に近い部位の切除，あるいは尿管の切断を伴う形成が必要な場合は尿管カテの挿入など，泌尿器科医と合同で手術的な治療を行うことが必要となる．尿管の閉塞を伴うような症例においては子宮内膜症による閉塞は尿管口に近い症例が多く，尿管膀胱新吻合が必要となる．子宮内膜症の病巣切除と腹腔内の観察は腹腔鏡手術が優れているが，尿管の操作に関してはNezhatや安藤らが報告しているように，かなり熟練した術者でないと腹腔鏡での施行は困難であり[8][9]，開腹手術と腹腔鏡手術との選択は，術者の技量による．

2．小骨盤腔外の子宮内膜症

1）横隔膜・肺の子宮内膜症の治療

治療法に関して現在コンセンサスはないが，胸腔鏡の普及により，繰り返し発症している症例に関しては，外科的診断と治療（切除）が基本となってきている[5]．

2）薬剤による治療法の施行に際して

子宮内膜症の発症した臓器にかかわらず，臨床症状の軽微なものや，手術による切除が困難であったり，治療による合併症が重篤であると判断された場合は薬剤による治療が主体となる．薬剤の種類は表2に示したが，GnRHアゴニスト，ダナゾール，およびピル（低容量，および中容量）が主たる薬剤である．しかし薬剤による治療により，横隔膜子宮内膜症では症状が悪化する症例と寛解した症例の両者が報告されていて，薬物治療施行の可否および有効性について結論は出ていない．また第一選択として薬剤治療を始める場合は，外科的診断をつけずに薬剤による治療を始めることになるので，薬剤による副作用と外科的手術の侵襲を評価し，また妊孕性や挙児希望を考慮しながら，治療法を慎重に決めていく必要がある．

近年では，腹腔鏡と胸腔鏡の普及に伴って，NSAIDsなどの鎮痛剤による経過観察が不可能な症例に対しては，外科的診断と切除による治療が第一選択となってきているのが現状である．

■■ ま と め

異所性子宮内膜症の発症部位は多岐にわたる．その発症機序として転移，化生あるいは胎生期組織遺残の可能性が考えられているが，男性の前立腺周囲においても異所性子宮内膜症が発症することから，化生および胎生期組織遺残が一つの要因であることは明らかである．

一方，左側の骨盤壁や直腸・S状結腸などの月経血が遺残しやすい部位が異所性子宮内膜症の好

発部位であることは，内膜組織の撒布も一つの原因になっていると推測される．異所性子宮内膜症は消化管や呼吸器という機能温存が必要な重要臓器に発症することが比較的多く，産婦人科医としては，ほかの診療科と合同して治療にあたることが望ましい．臨床症状が明らかであるときは外科的治療が優先されているが，薬剤による治療で寛解する症例報告もあり，治療方法は症例ごとに考慮する必要があるであろう．

文　献

1) Chapron C, Fauconnier A, Dubuisson JB, et al : Deep infiltrating endometriosis : relation between severity of dysmenorrhoea and extent of disease. Hum Reprod 18 : 760—766, 2003.
2) Abbott JA, Hawe J, Clayton RD, et al : The effects and effectiveness of laparoscopic excision of endometriosis : a prospective study with 2-5 year follow-up. Hum Reprod 18 : 1922—1927, 2003.
3) Chapron C, Liaras E, Fayet P, et al : Magnetic resonance imaging and endometriosis : deeply infiltrating endometriosis does not originate from the recto-vaginal septum. Gynecol Obstet Invest 53 : 204—208, 2002.
4) Suginami H : A reappraisal of the coelomic metaplasia theory by reviewing endometriosis occurring in unusual sites and instances. Am J Obstet Gynecol 165 : 214—218, 1991.
5) Korom S, Canyurt H, Missbach A, et al : Catamenial pneumothorax revisited : clinical approach and systematic review of the literature. J Thorac Cardiovasc Surg 128 : 502—508, 2004.
6) Alifano M, Roth T, Broet SC, et al : Catamenial pneumothorax : a prospective study. Chest 124 : 1004—1008, 2003.
7) Redwine DB, Wright JT : Laparoscopic treatment of complete obliteration of the cul-de-sac associated with endometriosis : long-term follow-up of en bloc resection. Fertil Steril 76 : 358—365, 2001.
8) Nezhat CH, Nezhat FR, Freiha F, et al : Laparoscopic vesicopsoas hitch for infiltrative ureteral endometriosis. Fertil Steril 71 : 376—379, 1999.
9) Andou M, Yoshioka T, Ikuma K : Laparoscopic ureteroneocystostomy. Obstet Gynecol 102 : 1183—1185, 2003.

〔シンポジウム1／子宮内膜症の病因に関する基礎研究〕

子宮内膜症関連遺伝子同定のための新しいアプローチ

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目 的

月経時に特異的に発現レベルが変動する子宮内膜遺伝子産物は，逆流月経内膜の異所性生着に引き続く子宮内膜症の発症，ならびに，反復する月経周期を通じて内膜症病変が増悪するメカニズムに関与すると考えられる．本研究では，*in vitro* 月経モデルおよびDNAマイクロアレイを用いた網羅的遺伝子解析により，子宮内膜症関連遺伝子を探索し同定することを目的とした．

方 法

患者の同意を得て摘出した良性疾患子宮の内膜より間質細胞を分離・純化した後，17beta-estradiolおよびprogesteroneの非存在下および存在下で2週間培養した．続いて前者は非存在下で(C群)，後者は性ステロイド存在下(EP群)と非存在下（withdrawal群，WD群）の2群に分けてさらに2日間培養した．定法により得られた各群のcRNAを約33,000の遺伝子解析が可能なGeneChip®（Affymetrix U133Set）に供し，発現の増強および低下を検討した．C群およびEP群に比較してin vitro月経モデルに相当するWD群において，その発現が2倍以上増強あるいは半分以下に減弱しているものをWD特異的変動遺伝子とした．また，それらの実際の変動をRT-PCRおよびrealtime PCRにて確認した．さらに，各月経周期の内膜組織および手術検体より同意の下に得られた子宮内膜症病変組織よりmRNAを抽出し，RT-PCRで対象とする遺伝子の発現レベルを解析した．

成 績

GeneChip解析で同定されたWD特異的変動遺伝子の中には，月経あるいは内膜症関連遺伝子としてすでに報告されているmonocyte chemotactic protein-1(MCP-1)，interleukin-8(IL-8)，matrix metalloproteinase-8（MMP-8）が含まれており，本法の戦略と方法の妥当性が示唆された．また，それらは，Gene chip解析と同様，RT-PCRあるいはreal-time PCRによる解析においても同様の挙動を示した．さらに，これらWD特異的変動遺伝子の中で，未知の遺伝子の断片がWD群に強く発現しており，これを一時的にEST-Xと呼称するが，これも同様の挙動をRT-PCRでも確認した．EST-Xは増殖期・分泌期に比べて月経時の正所性内膜に強く発現しているだけでなく，子宮内膜症病変においても，正所性内膜に比べてその発現レベルが上昇していた．

結 論

現在EST-Xの全長遺伝子クローニングを行っているが，本研究で用いた新しいアプローチにより，新規の内膜症関連遺伝子を同定し得る可能性が示された．

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Review Article

The present status of artificial oocyte activation in assisted reproductive technology

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Intracytoplasmic sperm injection (ICSI) is the most effective treatment for achieving fertilization in assisted reproductive technology (ART). However, fertilization failure occurs. The incidence of fertilization failure after ICSI is 1–5%. Approximately 50% of fertilization failure cases could be attributed to the abnormality of sperm factor. As the fertilization fails after ICSI using mature sperm, round spermatids and globozoospermia, artificial oocyte activation may provide a means of improving fertilization rates in such cases. The oocyte activation treatments used in clinical research include calcium (Ca) ionophore treatment, electrostimulation and strontium treatment. In terms of the efficiency of oocyte activation, electrostimulation and Ca ionophore gave better outcomes than strontium treatment. Strontium treatment causes Ca^{2+} oscillations in mice, so it has been viewed favorably. However, in human oocytes calcium oscillation has not been observed. The fertilization rate after ICSI was low in the case of globozoospermia and wiht round spermatids. Some cases of pregnancy were achieved by ICSI alone and oocyte activation methods were not essential in these cases. Among the various oocyte activation methods currently used, it should be noted that issues of genetic safety have not been addressed for the combined use of these oocyte activation methods. (Reprod Med Biol 2008; 7: 133–142)

Key words: calcium ionophore, electrostimulation, fertilization failure, intracytoplasmic sperm injection, oocyte activation.

INTRODUCTION

OOCYTE ACTIVATION IS the process by which oocytes arrested in metaphase II of meiosis are stimulated to resume meiosis.[1] This process is marked by pore formation and secretion in the cortical granules, and release of the second polar body. It is necessary for the initiation of fertilization and occurs when the sperm adheres to the cell membrane of the oocyte. However, it can also be triggered by chemical or physical stimulation in a process known as artificial oocyte activation.

Artificial oocyte activation has been used not only to study the mechanism of oocyte activation,[2] but also in studies of parthenogenesis[3] and nuclear transplantation.[4] Activation of oocytes is precipitated by various types of stimulation, including exposure to ultraviolet light,[5] changes in osmotic pressure,[6] treatment with calcium (Ca) ionophores,[3] electrostimulation,[7] and treatment with strontium,[8] puromycin[9] or cycloheximide.[5]

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Interestingly, the fertilization rate resulting from intracytoplasmic sperm injection (ICSI) is increased when the oocytes undergo artificial oocyte activation in hamsters[10,11] and humans.[12] This raises the possibility that artificial oocyte activation might be used to enhance fertilization rates alongside other techniques. As the fertilization fails after ICSI using mature sperm, immature male germ cells and round head sperm, artificial oocyte activation might provide a means of improving the fertilization rates in such cases.

FERTILIZATION BY INTRACYTOPLASMIC SPERM INJECTION

OOCYTE ACTIVATION OCCURS early in the process of fertilization and involves the resumption of meiosis of oocytes arrested at the metaphase stage of meiosis II. The details of the mechanism are not clear, but a transient increase in calcium ions ($[Ca^{2+}]_i$) inside the oocyte is known to play an important role.[13,14] In ICSI-assisted fertilization, the oocyte is activated by a sperm-derived oocyte activation factor (also known as sperm factor) after the injection.[14–16] A decrease or loss in activity of this factor is a possible cause of fertilization failure with ICSI. When this factor is completely

Table 1 Types of artificial oocyte activation methods and reported cases in clinical research

Types of artificial oocyte activation methods	Mechanism of oocyte activation	Cases of pregnancy and delivery in clinical research
Calcium ionophore†	Increase in Ca^{2+} permeability of cell membrane	+ (Hoshi *et al.* 1995 A23187[27])
Electrostimulation	Pore formation in cell membrane, entry of extracellular Ca^{2+}	+ (Yanagida *et al.* 1999[28])
Strontium	Release of endogenous Ca^{2+}	+ (Yanagida *et al.* 2006[29])
Ethanol	Increase in Ca^{2+} permeability of cell membrane	
Puromycin‡	Inhibition of protein synthesis	+ (Murase *et al.* 2004[30])
Cycloheximide	Inhibition of protein synthesis	

†Calcium ionophores include A23187 and ionomycin. ‡Puromycin is used in combination with A23187.

absent, oocyte activation does not occur and the oocyte remains in metaphase II even if ICSI is carried out. We are able to evaluate the ability of sperm to activate oocytes using mouse oocytes.[17,18] Immobilized sperm injected into the oocyte only undergo decondensation if the sperm nucleoprotein and the oocyte reduction mechanism are normal.[19] When the activity level of the sperm factor is reduced, the oocyte activation mechanism is stimulated at a low level, resulting in a reduction in the level of maturation-promoting factor (MPF) in the oocyte and in the release of the second polar body.[20] However, in some cases, MPF is resynthesized and the activation mechanism of the oocyte is halted. In these cases, premature chromatin condensation (PCC) sometimes occurs after the release of the second polar body and decondensation of the sperm injected into the ooplasm (metaphase III).

Although a transient increase in Ca ions in the oocyte is necessary for oocyte activation, subsequent Ca^{2+} oscillations are not absolutely required. In many oocyte activation methods, the oocyte is activated by inducing a single transient increase in the concentration of Ca^{2+}. Although the functional significance of Ca^{2+} oscillations is not clear, its continuation from the postfertilization to pronuclear fusion stages suggests a role in embryonic development.[21] Furthermore, in mice, parthenogenetic oocytes without Ca^{2+} oscillations develop into blastocysts with a smaller inner cell mass.[22] In addition, Ca^{2+} oscillations affect pronucleus formation,[23] the arrest of early embryo development[24] and post-implantation development.[25]

TYPES AND MECHANISMS OF ARTIFICIAL OOCYTE ACTIVATION

OOCYTE ACTIVATION REQUIRES either a transient increase in Ca^{2+} in the oocyte or a decline in MPF,[26] which normally follows such a Ca spike. Methods of artificial oocyte activation are usually based on one of these mechanisms (Table 1).

Calcium ionophores

Calcium ionophores, including A23187[1] and ionomycin,[31] are commonly used to induce oocyte activation. The first case in which the combined use of A23187 and ICSI resulted in a successful pregnancy and delivery was reported in 1995.[27] This case used the combination treatment to increase the fertilization rate and several subsequent cases have been reported in recent years.[32–36]

Mechanism

Calcium ionophores help activate the oocyte by increasing the Ca^{2+} permeability of the cell membrane, thereby letting extracellular Ca^{2+} flow into the cell.[37] The oocyte activating effect of A23187 is weakened by the presence of protein, including human serum albumin. When a strong effect is desired, a Ca^{2+}/Mg^{2+}-free culture medium should be used, and the osmotic pressure should be corrected using polyvinylpyrrolidone. The addition of albumin may be used to stop the ionophore treatment.

For human oocytes, serum-containing medium is preferred for its gentler effect on the oocyte. Following treatment with A23187, the Ca^{2+} concentration in the oocyte peaks after approximately 1 min and then gradually decreases.[37] The treatment causes a single transient increase in Ca ion concentration, but no Ca^{2+} oscillation.

Method

1. Dissolve A23187 (C7522; Sigma, St Louis, MO, USA) in dimethyl sulfoxide (DMSO) to make a 1 mmol/L stock solution. The stock solution should be kept frozen at −80°C.
2. Add 10 μL of the stock solution to 990 μL of culture medium (e.g. human tubal fluid [HTF]) and mix to make 10 μmol/L A23187 treatment solution. Use

the treatment solution as soon as possible after preparation and keep it protected from light exposure until use.
3. Add the A23187 treatment solution to the oocyte and place in a 37°C incubator for 5–15 min.
4. After treatment is completed, immediately wash the oocyte three times with standard culture medium and resume incubation.

When oocyte activation with ICSI alone fails, A23187 treatment may be carried out before or after ICSI to improve the fertilization rates. One report describes treating oocytes with A23187 approximately 30 min after performing ICSI.[27]

Electrostimulation

Electrostimulation is used in studies of parthenogenesis or for embryo cloning in the field of animal science. Fundamental research[11,38] and clinical studies[27] of electrostimulation with ICSI in human oocytes have been reported.

Mechanism

The oocyte is placed between two parallel electrode plates. The electric field generated by a direct current voltage causes charged proteins in the lipid bilayer of the cell membrane to move, thereby forming pores in the membrane.[39] Extracellular Ca^{2+} in the culture medium flow into the oocyte through these pores, transiently elevating the interior Ca^{2+} concentration and activating the oocyte. The lower the electrolyte concentration in the culture medium, the more pore formation is stimulated. The pores are thought to take 10–40 min at 37°C to repair, and longer when the temperature is lower.[40] The concentration of Ca^{2+} is elevated immediately after the application of the stimulus, peaks within 1 min, and then gradually decreases and returns to the original level in approximately 5 min, without subsequent oscillation.[11,47] When electrostimulation was applied repeatedly to imitate Ca^{2+} oscillations in unfertilized rabbit oocytes, parthenogenesis occurred and fetuses were obtained.[41]

Method

The actual process of electrostimulation is described as follows:

1. Fill the electrode chamber with Zimmerman solution[39] or Dulbecco's phosphate buffered saline (D-PBS).[11]
2. Place the oocyte (or multiple oocytes) between the electrodes in the chamber.
3. Apply a rectangular wave of electrostimulation using an electroporator (e.g. apply one pulse of 100–150 V for 50–100 μsec when the distance between the electrodes is 1 mm).
4. Incubate the oocyte in standard culture medium.

The conditions for electrostimulation vary according to the electroporator and chamber type (the distance between the electrodes and the material of the electrodes). Conditions must be set in advance using a 1-day-old oocyte. Although D-PBS is used as the pulsing medium in the above method, 0.3 mol/L mannitol solution (containing 100 μmol/L $CaCl_2$ and 100 μmol/L $MgCl_2$) is more typically used in basic research. Because D-PBS contains more electrolytes, the electric current created by electrostimulation would normally be a problem, but the electric resistance between the electrodes is approximately 13 KΩ or higher, and the electric current created is very weak, at approximately 1 mA. Thus, out of concern that changes in the composition of the culture medium might damage the cell, D-PBS is used.

Strontium treatment method

Because strontium treatment has been shown to cause oocyte activation accompanied with Ca^{2+} oscillations in mouse models,[42] this method is preferred when embryonic development is desired. In contrast, only the initial transient increase in cytoplasmic Ca^{2+} concentration is achieved with A23187 treatment and electrostimulation.[37] Another characteristic of strontium treatment is varied efficacy of oocyte activation depending on the species.[29,43–46] It is most effective in mice, but its efficacy has not been sufficiently confirmed in humans. Nonetheless, cases of pregnancy and delivery have been reported in clinical research applications.[29]

Mechanism

Sr^{2+} induced Ca^{2+} transients in an activated oocyte.[47] However, the mechanism by which Sr^{2+} induces Ca^{2+} oscillations in an oocyte remains unclear. Recently, Ca^{2+} oscillations induced by Sr^{2+} were mediated through inositol trisphosphate receptors.[48] Sr^{2+} is thought to move into the oocyte down the concentration gradient, causing Ca to be released from the endoplasmic reticulum.

Method

1. Dissolve $SrCl_2 \cdot 6H_2O$ (Sigma) in distilled water to make 1 mmol/L Sr^{2+} stock solution and store in the deep freezer.
2. Add 20 μL of the stock solution to 1 mL of a Ca^{2+}-free culture medium (such as HTF) to make 20 mmol/L strontium treatment solution.

3. Place the oocyte into the strontium treatment solution and incubate it for 120 min in an incubator.
4. Transfer the oocyte into standard culture medium and incubate.

The impact of strontium treatment on the chromosomes of gametes has been studied in mice and no effect has been reported.[43]

CLINICAL APPLICATION OF OOCYTE ACTIVATION METHODS

Cases of fertilization failure with ICSI

WHEN ICSI DOES not result in oocyte activation, treatments that artificially activate oocytes are used concomitantly with ICSI to promote fertilization. When ICSI is carried out, the incidence of fertilization failure is 1–5%.[30–34] The number of oocytes used in ICSI is closely associated with the fertilization rate.[32] The incidence of fertilization failure is 13.3% when ICSI is carried out with one oocyte, but decreases to 3.1% with two oocytes and 1% or lower with three or more oocytes. There was no fertilization failure with six or more oocytes.[32] When a small number of oocytes are used in ICSI, the high incidence of failure can be attributed to the poor quality of the collected oocytes or to technical factors (e.g. if a device malfunctioned during the initial ICSI, it would be fixed). Fertilization failure was observed in 5.6% (100/1779) of treatment cycles of ICSI. When fertilization failed in the initial ICSI, the probability of fertilization failure in the second ICSI was 13% and the incidence of such cases is 0.7% in all treatment cycles.[52]

Because the steps between capacitation and sperm–egg fusion are bypassed in ICSI, the problem must lie in later steps of the fertilization process. In particular, abnormalities in oocyte activation, decondensation of the sperm head or pronucleus formation may play a role in fertilization failure. The mechanism of oocyte activation in ICSI is thought to involve the action of phospholipase C zeta (PLCzeta), which is present in the sperm.[53] When the activity of this factor is low or lacking, fertilization fails. In addition, after fertilization, the sperm needs to undergo decondensation, which depends on the condition of the sperm nucleoprotein as well as the reduction system in the oocyte.[19] For example, decondensation can be impaired when the zinc supply from the seminal plasma is decreased because of a disorder, such as prostatitis, or when a large number of disulfide bonds are formed in the nucleoproteins.[56] The sperm aster also plays an important role in the fusion of male and female pronuclei. Abnormalities in the sperm centrosome that comprise the sperm aster can impair decondensation.[57] When oocytes that were not successfully fertilized following ICSI were observed using chromatin staining, oocyte activation failed to occur in 70% of the oocytes, even though the sperm was successfully inserted into the oocyte.[52] Moreover, when ICSI was carried out using sperm from volunteers whose sperm had been shown to be capable of fertilizing oocytes, fertilization was successful in 70% of the cases. Therefore, for approximately 50% of the oocytes that were not fertilized following ICSI, the failure of fertilization could be attributed to the sperm.[52]

If abnormalities in oocyte activation factor activity are the cause of fertilization failure, the combined use of oocyte activation treatments and ICSI can be considered. However, if the source of the problem lies elsewhere, there is currently no treatment method. The recurrence rate of fertilization failure following ICSI is 13%, which means that in many cases, fertilized oocytes can be obtained from a second round of ICSI.[52] When the risk of fertilization failure is high, however, an oocyte activation treatment combined with ICSI has been used. The oocyte activation treatments used in clinical research include Ca ionophore treatment, electrostimulation and strontium treatment. Table 2 shows the results of clinical research on the combined use of oocyte activation and ICSI in patients with fertilization failure and low fertilization cases following an ICSI attempt, excluding cases using spermatid and those with globozoospermia. We reported the first case of pregnancy and delivery after ICSI with electrostimulation. Since then there have been 10 delivery cases using the same treatment as the fertilization failure case. In terms of the efficiency of oocyte activation, electrostimulation and A23187 gave better outcomes than strontium treatment. Strontium treatment showed variable efficacy. This treatment was developed in studies using mice and has been viewed favorably because it causes Ca^{2+} oscillations and is similar to the physiological stimulus that activates oocytes.[41] However, in human oocytes, Ca^{2+} oscillations have not been observed following treatment and its role in human oocyte activation is not clear.[29] For clinical research, methods with known mechanisms should be selected. Chromosomal analysis of oocytes fertilized using both ICSI and oocyte activation did not show an increased risk from electrostimulation[36] and A23187 + puromycin.[59]

The timing of electrostimulation relative to ICSI is another important factor to consider. When electrostimulation is carried out prior to ICSI, the oocyte tends to

Table 2 Reports on the combined use of oocyte activation methods and intracytoplasmic sperm injection†

Reference	Indication	Types of oocyte activation methods	Cases of pregnancy and delivery
Tesarik *et al.* (1994)[12]	Fresh oocytes	A23187	Research‡
Hoshi *et al.* (1995)[27]	Low fertilization	A23187	Two cases of pregnancy
Yanagida *et al.* (1999)[28]	Fertilization failure	Electrical stimulation	One case of pregnancy and delivery
Yamano *et al.* (2000)[57]	1-day-old unfertilized oocytes	A23187 + puromycin	Research‡
Nakagawa *et al.* (2001)[58]	1-day-old unfertilized oocytes	A23187 + puromycin	Research‡
Tesarik *et al.* (2002)[59]	ICSI patients	Mechanical	One case of pregnancy and delivery
Eldar-Geva *et al.* (2003)[42]	Fertilization failure	A23187	One case of pregnancy and delivery
Murase *et al.* (2004)[60]	Fertilization failure	A23187 + puromycin	One case of pregnancy and delivery
Ebner *et al.* (2004)[60]	Fertilization failure	Mechanical	One case of pregnancy and delivery
Chi *et al.* (2004)[11]	Low fertilization	A23187	One case of pregnancy and delivery
Heindryckx *et al.* (2005)[44]	Fertilization failure	A23187	Three cases of pregnancy
Lu *et al.* (2006)[61]	1-day-old unfertilized oocytes	A23187 + puromycin	Research‡
Manipalviratn *et al.* (2006)[62]	1-day-old unfertilized oocytes	Electrostimulation	Research‡
Ahmady *et al.* (2007)[55]	Immotile testicular sperm	A23187	One case of pregnancy and delivery
Moaz *et al.* (2006)[63]	Low fertilization and fertilization failure	Ionomycin	Research‡
Yanagida *et al.* (2006)[29]	Low fertilization	Strontium	One case of pregnancy and delivery
Nasr-Esfahani *et al.* (2008)[56]	Low fertilization (teratozoospermia)	Ionomycin	Two cases of pregnancy

†Excluding cases using spermatids and globozoospermia. ‡'Research' means that this report was carried out for the purpose of research not a therapeutic purpose. ICSI, intracytoplasmic sperm injection.

degenerate. It is possible that the cell membrane is not sufficiently repaired after electrostimulation[40] and is further damaged by ICSI. Because the cell membrane can also be damaged by Ca ionophore treatment, we treat oocytes with Ca ionophore 30 min after ICSI. In addition, in mice, when the sperm is present during electrostimulation, the incidence of chromosomal aberration (structural abnormality) in the sperm is approximately 50% higher. In such cases, the chromosomes of the oocyte were unaffected. In contrast, when electrostimulation was carried out 30–60 min before ICSI, there were no chromosomal abnormalities, but the oocyte still tends to be damaged.

Special cases of ICSI include cases of round-headed spermatozoa (globozoospermia) and the use of spermatids. Round-headed spermatozoa possess round heads lacking an acrosome.[65] In spermatozoa, the level of sperm factor appears to be low and there are reported cases of fertilization failure associated with it, for which clinical application of ICSI has been reported (Table 3). The reported fertilization rates were 0–42% with ICSI alone and approximately 70% in conjunction with oocyte activation methods. With round-headed spermatozoa, many cases of pregnancy were achieved by ICSI alone[66–68,71–74,77] and oocyte activation methods were not essential. From clinical research using round spermatids, 14 cases of pregnancy have been reported to date (Table 4).[78,79,81,82,84–86,93,94] Because the activity level of the sperm factor is low in human round spermatids, oocyte activation in conjunction with ICSI has been considered. However, the reported fertilization rates were between 16 and 69% with ICSI alone.[78,79,81–89,92,95,96] Oocyte activation methods were used along with ICSI in a small number of reported cases and the fertilization rate was approximately 40%.[80,81] In our experience, the fertilization rate with round spermatids was 17%.[97] Elongated spermatids seem to have a similar oocyte activation capacity as mature sperm.

ASSISTED ACTIVATION

ASSISTED ACTIVATION IS based on the idea of using oocyte activation methods in conjunction with ICSI in patients without fertilization failure. These patients do not exhibit a low fertilization rate, but this approach is used to further raise the fertilization rate and increase the number of embryos obtained. As this method works on the oocytes that are still unfertilized after ICSI, the number of fertilized oocytes should increase. However, fertilized oocytes would also undergo

Table 3 Reported cases of intracytoplasmic sperm injection for globozoospermia

Reference	Oocyte activation treatment	Fertilization rate (%)	Cases of pregnancy and delivery
Liu *et al.* (1995)[66]	None	–	Two cases of pregnancy
Trokoudes *et al.* (1995)[67]	None	50	One case of pregnancy
Bourne *et al.* (1995)[68]	None	–	None
Battaglia *et al.* (1997)[69]	None	10	None
	A23187	75	None
Rybouchkin *et al.* (1997)[70]	A23187	–	One case of pregnancy
Stone *et al.* (2000)[71]	None	10–42	One case of pregnancy and delivery
Kim *et al.* (2001)[72]	A23187	60	One case of pregnancy and delivery
Zeyneloglu *et al.* (2002)[73]	None	31	One case of pregnancy and delivery
Nardo *et al.* (2002)[74]	None	–	One case of pregnancy and delivery
Kilani *et al.* (2004)[75]	None	38	One case of pregnancy and delivery
Heindryckx *et al.* (2005)[34]	A23187	71–77	Five cases of pregnancy
Khalili *et al.* (2007)[76]	None	0 (four cases)	None
Dirican *et al.* (2007)[77]	None	9, 33 (two cases)	Two cases of pregnancy and delivery

Table 4 Reports on round spermatid injection

Reference	Oocyte activation treatment	Fertilization rate (%)	Cases of pregnancy and delivery
Tesarik *et al.* (1995,96)[78,79]	None	36	Two cases of pregnancy and delivery
Tanaka *et al.* (1996)[80]	Electrostimulation	42	One case of pregnancy
Vanderzwalmen *et al.* (1997)[81]	None	16	None
	A23187	36	One case of pregnancy
Antinori *et al.* (1997)[82]	None	56	Two cases of pregnancy
Yamanaka *et al.* (1997)[83]	None	69	None
Amer *et al.* (1997)[84]	None	25	Four cases of pregnancy
Kahraman *et al.* (1998)[85]	None	26	One case of pregnancy
Barak *et al.* (1998)[86]	None	27	One case of pregnancy and delivery
Al-Hasani *et al.* (1999)[87]	None	18	None
Ghazzawi *et al.* (1999)[88]	None	22	None
Levran *et al.* (2000)[89]	None	44.9	None
Vicdan *et al.* (2001)[90]	None	–	None (0/6)
Khalili *et al.* (2002)[91]	None	–	None (0/7)
Sousa *et al.* (2002)[92]	None	16	None (0/33)
Saremi *et al.* (2002)[93]	None	–	One case of pregnancy and delivery
Amarin *et al.* (2002)[94]	None	–	One case of pregnancy
Ulug *et al.* (2003)[95]	None	42	None
Benkhalifa *et al.* (2004)[96]	None	36	None

treatment. Because the techniques of oocyte activation are still at the clinical research stage, they should not be used for such a purpose. When two or fewer oocytes are used in ICSI, the risk of fertilization failure is higher. When oocyte activation methods were concomitantly used with ICSI in such cases, the fertilization rate, embryonic development rate and pregnancy rate did not show any significant difference and, therefore, these methods were evaluated to be ineffective.[32]

RESCUE ACTIVATION

IN RESCUE ACTIVATION, oocyte activation is used to promote fertilization in oocytes that remain unfertilized following ICSI when the fertilization status is evaluated. The time interval between the collection of the oocyte and the oocyte activation treatment is important. Ontogenesis cannot be expected after aging of the oocyte. With 1-day-old oocytes, the pregnancy rate is extremely

low. When rescue activation was carried out for such oocytes in 52 cases, the fertilization rate was 78% and two cases of pregnancy were obtained, but both resulted in miscarriage.[52] Therefore, this method should not be used clinically for 1-day-old oocytes.[98,99] Rescue ICSI 6 h after insemination has already been shown to be effective in cases of fertilization failure with *in vitro* fertilization.[100] In the same way, rescue activation should be carried out within 6 h after ICSI. When oocyte activation does not occur following ICSI, the head of the injected sperm may undergo decondensation and PCC may occur over time. Premature chromatin condensation is the abnormal condensation of chromatin and can induce damage in the chromosome or DNA. Therefore, an evaluation of fertilization should be done before PCC takes place.[101] Because the incidence of PCC is elevated starting 4 h later when oocyte activation does not occur following ICSI, it is recommended that the fertilization status be evaluated and oocyte activation treatment carried out on unfertilized oocytes within 4 h.[102] If at least one fertilized oocyte is found at the evaluation, oocyte activation treatment is not necessary.

CONCLUSION

ARTIFICIAL OOCYTE ACTIVATION is frequently used in basic research in the field of reproductive technology. Oocyte activation occurs at the initial stage of fertilization and is essential for fertilization. In some cases of fertilization failure with ART, oocyte activation did not occur because of abnormalities in the sperm-derived oocyte activation factor. In 50% of the unfertilized oocytes following ICSI, the failure can be attributed to problems with the sperm.[52] When fertilization failure is predicted with ICSI, oocyte activation methods can be used along with ICSI in a clinical research setting. Oocyte activation methods can also be used as a rescue procedure when fertilization failure is detected within 4 h of ICSI.[101] Among the various oocyte activation methods currently used, Ca ionophore treatment is the easiest to use. However, it should be noted that issues of genetic safety and abnormal imprinting have not been addressed for the combined use of these oocyte activation methods.[102]

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REFERENCES

1 Carroll J. The initiation and regulation of Ca^{2+} signalling at fertilization in mammals. *Seminars Cell Dev Biol* 2001; **12**: 37–43.
2 Schuetz AW. Cytoplasmic activation of starfish oocytes by sperm and divalent ionophore A-23187. *J Cell Biol* 1975; **66**: 86–94.
3 Steinhardt RA, Epel D, Carroll EJ Jr, Yanagimachi R. Is calcium ionophore a universal activator for unfertilised eggs? *Nature* 1974; **252**: 41–43.
4 Wakayama T, Perry ACF, Zuccotti M, Johnson KR, Yanagimachi R. Full-term development of mice from enucleated oocytes injected with cumulus cell nuclei. *Nature* 1998; **394**: 369–374.
5 Bradshaw J, Jung T, Fulka J Jr, Moor RM. UV irradiation of chromosomal DNA and its effect upon MPF and meiosis in mammalian oocytes. *Mol Reprod Dev* 1995; **41**: 503–512.
6 Mahowald AP, Goralski TJ, Caulton JH. In vitro activation of Drosophila eggs. *Dev Biol* 1983; **98**: 437–445.
7 Prather RS, Eichen PA, Nicks DK, Peters MS. Artificial activation of porcine oocytes matured in vitro. *Mol Reprod Dev* 1991; **28**: 405–409.
8 Whittingham DG, Siracusa G. The involvement of calcium in the activation of mammalian oocytes. *Exp Cell Res* 1978; **113**: 311–317.
9 Siracusa G, Whittingham DG, Molinaro M, Vivarelli E. Parthenogenetic activation of mouse oocytes induced by inhibitors of protein synthesis. *J Embryol Exp Morphol* 1978; **43**: 157–166.
10 Hoshi K, Yanagida K, Sato A. Pretreatment of hamster oocytes with Ca^{2+} ionophore to facilitate fertilization by ooplasmic micro injection. *Hum Reprod* 1992; **7**: 1992.
11 Yanagida K, Katayose H, Hoshi K, Yazawa H, Sato A. Effect of electrical stimulation on oocyte activation after intracytoplasmic sperm injection. *J Mamm Ova Res* 1997; **14**: 132–138.
12 Tesarik J, Testart J. Treatment of sperm-injected human oocytes with Ca^{2+} ionophore supports the development of Ca^{2+} oscillations. *Biol Reprod* 1994; **51**: 385–391.
13 Miyazaki S, Shirakawa H, Nakada H, Honda Y. Essential role of the inositol 1,4,5-triphosphate receptor/Ca^{2+} release channel in Ca^{2+} waves and Ca^{2+} oscillations at fertilization of mammalian eggs. *Dev Biol* 1993; **158**: 62–78.
14 Swann K. A cytosolic sperm factor stimulates repetitive calcium increases and mimics fertilization in hamster eggs. *Development* 1990; **110**: 1295–1302.
15 Stice SL, Robl JM. Activation of mammalian oocytes by a factor obtained from rabbit sperm. *Mol Reprod Dev* 1990; **25**: 272–280.
16 Tesarik J, Sousa M, Testart J. Human oocyte activation after intracytoplasmic sperm injection. *Hum Reprod* 1994; **9**: 511–518.
17 Rybouchkin A, Dozortsev D, de Sutter P, Qian C, Dhont M. Intracytoplasmic injection of human spermatozoa into

mouse oocytes: a useful model to investigate the oocyte activating capacity and the karyotype of human spermatozoa. *Hum Reprod* 1995; **10**: 1130–1135.

[18] Araki Y, Yoshizawa M, Abe H, Murase Y, Araki Y. Use of mouse oocytes to evaluate the ability of human sperm to activate oocytes after failure of activation by intracytoplasmic sperm injection. *Zygote* 2004; **12**: 111–116.

[19] Yanagimachi R. Mammalian fertilization. In: Knobil E, Neill JD, eds. *The Physiology of Reproduction*, 2nd edn. Raven Press, New York, 1994; 189–317.

[20] Moor RM. Regulation of the meiotic cycle in oocytes of domestic mammals. *Ann NY Acad Sci* 1988; **541**: 248–258.

[21] Jones K, Carroll J, Merriman J, Whittingham D, Kono T. Repetitive sperm-induced Ca^{2+} transients in mouse oocytes are cell cycle dependent. *Development* 1995; **121**: 3259–3266.

[22] Bos-Mikich A, Whittingham DG, Jones KT. Meiotic and mitotic Ca^{2+} oscillations affect cell composition in resulting blastocysts. *Dev Biol* 1997; **182**: 172–179.

[23] Lawrence Y, Ozil J, Swann K. The effects of a Ca^{2+} chelator and heavy-metal-ion chelators upon Ca^{2+} oscillations and activation at fertilization in mouse eggs suggest a role for repetitive Ca^{2+} increases. *Biochem J* 1998; **335**: 335–342.

[24] Gordo A, Rodrigues P, Kurokawa M *et al.* Intracellular calcium oscillations signal apoptosis rather than activation in in vitro aged mouse eggs. *Biol Reprod* 2002; **66**: 1828–1837.

[25] Ozil J, Huneau D. Activation of rabbit oocytes: the impact of the Ca^{2+} signal regime on development. *Development* 2001; **128**: 917–928.

[26] Gerhart J, Wu M, Kirschner M. Cell cycle dynamics of an M-phase-specific cytoplasmic factor in Xenopus laevis oocytes and eggs. *J Cell Biol* 1984; **98**: 1247–1255.

[27] Hoshi K, Yanagida K, Yazawa H, Katayose H, Sato A. Intracytoplasmic sperm injection using immobilized or motile human spermatozoon. *Fertil Steril* 1995; **63**: 1241–1245.

[28] Yanagida K, Katayose H, Yazawa H *et al.* Successful fertilization and pregnancy following ICSI and electrical oocyte activation. *Hum Reprod* 1999; **14**: 1307–1311.

[29] Yanagida K, Morozumi K, Katayose H, Sato A. Successful pregnancy after ICSI with strontium oocyte activation in low rates of fertilization. *Reprod Biomed Online* 2006; **13**: 2006.

[30] Murase Y, Araki Y, Mizuno S *et al.* Pregnancy following chemical activation of oocytes in a couple with repeated failure of fertilization using ICSI: case report. *Hum Reprod* 2004; **19**: 1604–1607.

[31] Navara CS, First NL, Schatten G. Microtubule organization in the cow during fertilization, polyspermy, parthenogenesis, and nuclear transfer: the role of the sperm aster. *Dev Biol* 1994; **162**: 29–40.

[32] Eldar-Geva T, Brooks B, Margalioth EJ, Zylber-Haran E, Gal M, Silber SJ. Successful pregnancy and delivery after calcium ionophore oocyte activation in a normozoospermic patient with previous repeated failed fertilization after intracytoplasmic sperm injection. *Fertil Steril Supplement* 2003; **3**: 1656–1658.

[33] Chi HJ, Koo JJ, Song SJ, Lee JY, Chang SS. Successful fertilization and pregnancy after intracytoplasmic sperm injection and oocyte activation with calcium ionophore in a normozoospermic patient with extremely low fertilization rates in intracytoplasmic sperm injection cycles. *Fertil Steril* 2004; **82**: 475–477.

[34] Heindryckx B, Van der Elst J, De Sutter P, Dhont M. Treatment option for sperm- or oocyte-related fertilization failure: assisted oocyte activation following diagnostic heterologous ICSI. *Hum Reprod* 2005; **20**: 2237–2241.

[35] Ahmady A, Michael E. Successful pregnancy and delivery following intracytoplasmic injection of frozen-thawed nonviable testicular sperm and oocyte activation with calcium ionophore. *J Androl* 2007; **28**: 13–14.

[36] Nasr-Esfahani MH, Razavi S, Javdan Z, Tavalaee M. Artificial oocyte activation in severe teratozoospermia undergoing intracytoplasmic sperm injection. *Fertil Steril* 2008; **16** [Epub ahead of print].

[37] Swann K, Ozil JP. Dynamics of the calcium signal that triggers mammalian egg activation. *Int Rev Cytol* 1994; **152**: 183–222.

[38] Zhang J, Wang CW, Blaszcyzk A *et al.* Electrical activation and in vitro development of human oocytes that fail to fertilize after intracytoplasmic sperm injection. *Fertil Steril* 1999; **72**: 509–512.

[39] Zimmerman U, Vienken J. Electric field-induced cell-to-cell fusion. *J Membr Biol* 1982; **67**: 165–182.

[40] Bates GW, Saunders A, Sowers AE. Electrofusion. In: Sowers AE, ed. *Cell Fusion*. Plenum Press, New York, 1987; 367–395.

[41] Ozil JP. The parthenogenetic development of rabbit oocytes after repetitive pulsatile electrical stimulation. *Development* 1990; **109**: 117–127.

[42] Cheek TR, McGuinness OM, Vincent C, Moreton RB, Berridge MJ, Johnson MH. Fertilisation and thimerosal stimulate similar calcium spiking patterns in mouse oocytes but by separate mechanisms. *Development* 1993; **119**: 179–189.

[43] Kato M, Ishikawa A, Hochi S, Hirabayashi M. Effect of activation regimens for rat oocytes on full-term development after round spermatid injection. *Contemp Top Lab Anim Sci* 2004; **43**: 13–15.

[44] Tateno H, Kamiguchi Y. Parthenogenetic activation of Chinese hamster oocytes by chemical stimuli and its cytogenetic evaluation. *Mol Reprod Dev* 1997; **47**: 72–78.

[45] Méo SC, Yamazaki W, Leal CL, de Oliveira JA, Garcia JM. Use of strontium for bovine oocyte activation. *Theriogenology* 2005; **63**: 2089–2102.

[46] Okada K, Miyano T, Miyake M. Activation of pig oocytes by intracytoplasmic injection of strontium and barium. *Zygote* 2003; **11**: 159–165.

[47] Kono T, Jones KT, Mikich AB, Whittingham DG, Carroll J. A cell cycle-associated change in Ca^{2+} releasing activity leads to

the generation of Ca^{2+} transients in mouse embryos during the first mitotic division. *J Cell Biol* 1996; **132**: 915–923.

[48] Zhang D, Pan L, Yang LH, He XK, Huang XY, Sun FZ. Strontium promotes calcium oscillations in mouse meiotic oocytes and early embryos through InsP3 receptors, and requires activation of phospholipase and the synergistic action of InsP3. *Hum Reprod* 2005; **20**: 3053–3061.

[49] Morozumi K, Tateno H, Yanagida K, Katayose H, Kamiguchi Y, Sato A. Chromosomal analysis of mouse spermatozoa following physical and chemical treatments that are effective in inactivating HIV. *Zygote* 2004; **12**: 339–344.

[50] Moomjy M, Sills ES, Rosenwaks Z, Palermo GD. Implications of complete fertilization failure after intracytoplasmic sperm injection for subsequent fertilization and reproductive outcome. *Hum Reprod* 1998; **13**: 2212–2216.

[51] Ludwig M, Strik D, Al-Hasani S, Diedrich K. No transfer in a planned ICSI cycle: we cannot overcome some basic rules of human reproduction. *Eur J Obstet Gynecol Reprod Biol* 1999; **87**: 3–11.

[52] Yanagida K. Complete fertilization failure in ICSI. *Hum Cell* 2004; **17**: 187–193.

[53] Liu J, Nagy Z, Joris H *et al*. Analysis of 76 total fertilization failure cycles out of 2732 intracytoplasmic sperm injection cycles. *Hum Reprod* 1995; **10**: 2630–2636.

[54] Esfandiari N, Javed MH, Gotlieb L, Casper RF. Complete failed fertilization after intracytoplasmic sperm injection – analysis of 10 years' data. *Int J Fertil Womens Medical* 2005; **50**: 187–192.

[55] Saunders CM, Larman MG, Parrington J *et al*. PLC zeta: a sperm-specific trigger of Ca^{2+} oscillations in eggs and embryo development. *Development* 2002; **129**: 3533–3544.

[56] Kuvist U. Importance of spermatozoal zinc as temporary inhibitor of sperm nuclear chromatin decondensation ability in man. *Acta Phisiol Scand* 1980; **109**: 79–84.

[57] Terada Y, Nakamura S, Simerly C *et al*. Centrosomal function assessment in human sperm using heterologous ICSI with rabbit eggs: a new male factor infertility assay. *Mol Reprod Dev* 2004; **67**: 360–365.

[58] Yamano S, Nakagawa K, Nakasaka H, Aono T. Fertilization failure and oocyte activation. *J Med Invest* 2000; **47**: 1–8.

[59] Nakagawa K, Yamano S, Moride N, Yamashita M, Yoshizawa M, Aono T. Effect of activation with Ca ionophore A23187 and puromycin on the development of human oocytes that failed to fertilize after intracytoplasmic sperm injection. *Fertil Steril* 2001; **76**: 148–152.

[60] Tesarik J, Rienzi L, Ubaldi F, Mendoza C, Greco E. Use of a modified intracytoplasmic sperm injection technique to overcome sperm-borne and oocyte-borne oocyte activation failures. *Fertil Steril* 2002; **78**: 619–624.

[61] Ebner T, Moser M, Sommergruber M, Jesacher K, Tews G. Complete oocyte activation failure after ICSI can be overcome by a modified injection technique. *Hum Reprod* 2004; **19**: 1837–1841.

[62] Lu Q, Zhao Y, Gao X *et al*. Combination of calcium ionophore A23187 with puromycin salvages human unfertilized oocytes after ICSI. *Eur J Obstet Gynecol Reprod Biol* 2006; **126**: 72–76.

[63] Manipalviratn S, Ahnonkitpanit V, Numchaistika P, Chompurat D, Pansatha J, Suwajanakorn S. Results of direct current electrical activation of failed-to-fertilize oocytes after intracytoplasmic sperm injection. *J Reprod Med* 2006; **51**: 493–499.

[64] Moaz MN, Khattab S, Foutouh IA, Mohsen EA. Chemical activation of oocytes in different types of sperm abnormalities in cases of low or failed fertilization after ICSI: a prospective pilot study. *Reprod Biomed Online* 2006; **13**: 791–794.

[65] Kullander S, Rausing A. On round-headed human spermatozoa. *Int J Fertil* 1975; **20**: 33–40.

[66] Liu J, Nagy Z, Joris H, Tournaye H, Devroey P, Van Steirteghem A. Successful fertilization and establishment of pregnancies after intracytoplasmic sperm injection in patients with globozoospermia. *Hum Reprod* 1995; **10**: 626–629.

[67] Trokoudes KM, Danos N, Kalogirou L *et al*. Pregnancy with spermatozoa from a globozoospermic man after intracytoplasmic sperm injection treatment. *Hum Reprod* 1995; **10**: 880–882.

[68] Bourne H, Liu DY, Clarke GN, Baker HW. Normal fertilization and embryo development by intracytoplasmic sperm injection of round-headed acrosomeless sperm. *Fertil Steril* 1995; **63**: 1329–1332.

[69] Battaglia DE, Koehler JK, Klein NA, Tucker MJ. Failure of oocyte activation after intracytoplasmic sperm injection using round-headed sperm. *Fertil Steril* 1997; **68**: 118–122.

[70] Rybouchkin A, Van Der Elst J, De Sutter P, Dhont M. 'Globe-headed spermatozoa' and ICSI. *Fertil Steril* 1998; **69**: 361–362.

[71] Stone S, O'Mahony F, Khalaf Y, Taylor A, Braude P. A normal livebirth after intracytoplasmic sperm injection for globozoospermia without assisted oocyte activation: case report. *Hum Reprod* 2000; **15**: 139–141.

[72] Kim ST, Cha YB, Park JM, Gye MC. Successful pregnancy and delivery from frozen–thawed embryos after intracytoplasmic sperm injection using round-headed spermatozoa and assisted oocyte activation in a globozoospermic patient with mosaic Down syndrome. *Fertil Steril* 2001; **75**: 445–447.

[73] Zeyneloglu HB, Baltaci V, Duran HE, Erdemli E, Batioglu S. Achievement of pregnancy in globozoospermia with Y chromosome microdeletion after ICSI. *Hum Reprod* 2002; **17**: 1833–1836.

[74] Nardo LG, Sinatra F, Bartoloni G, Zafarana S, Nardo F. Ultrastructural features and ICSI treatment of severe teratozoospermia: report of two human cases of globozoospermia. *Eur J Obstet Gynecol Reprod Biol* 2002; **104**: 40–42.

[75] Kilani Z, Ismail R, Ghunaim S *et al*. Evaluation and treatment of familial globozoospermia in five brothers. *Fertil Steril* 2004; **82**: 1436–1439.

[78] Khalili MA, Kalantar SM, Vahidi S, Ghafour-Zadeh M. Failure of fertilization following intracytoplasmic injection of round-headed sperm. *Ann Saudi Med* 1998; **18**: 408–411.
[79] Dirican EK, Isik A, Vicdan K, Sozen E, Suludere Z. Clinical pregnancies and livebirths achieved by intracytoplasmic injection of round headed acrosomeless spermatozoa with and without oocyte activation in familial globozoospermia: case report. *Asian J Androl* 2007: [Epub ahead of print].
[80] Tesarik J, Mendoza C, Testart J. Viable embryos from injection of round spermatids into oocytes. *N Engl J Med* 1995; **333**: 525.
[81] Tesarik J, Mendoza C. Spermatid injection into human oocytes. I. Laboratory techniques and special features of zygote development. *Hum Reprod* 1996; **11**: 772–779.
[82] Tanaka A, Nagayoshi M, Awata S *et al.* Clinical evaluation of round spermatid injection (ROSI) into human oocytes. *Fertil Steril* 1996; **Suppl.**: S99.
[83] Vanderzwalmen P, Zech H, Birkenfeld A *et al.* Intracytoplasmic injection of spermatids retrieved from testicular tissue: influence of testicular pathology, type of selected spermatids and oocyte activation. *Hum Reprod* 1997; **12**: 1203–1213.
[84] Antinori S, Versaci C, Dani G, Antinori M, Pozza D, Selman HA. Fertilization with human testicular spermatids: four successful pregnancies. *Hum Reprod* 1997; **12**: 286–291.
[85] Yamanaka K, Sofikitis NV, Miyagawa I *et al.* Ooplasmic round spermatid nuclear injection procedures as an experimental treatment for nonobstructive azoospermia. *J Assist Reprod Genet* 1997; **14**: 55–62.
[86] Amer M, Soliman E, el-Sadek M, Mendoza C, Tesarik J. Is complete spermiogenesis failure a good indication for spermatid conception? *Lancet* 1997; **350**: 116.
[87] Kahraman S, Polat G, Samli M *et al.* Multiple pregnancies obtained by testicular spermatid injection in combination with intracytoplasmic sperm injection. *Hum Reprod* 1998; **13**: 104–110.
[88] Barak Y, Kogosowski A, Goldman S, Soffer Y, Gonen Y, Tesarik J. Pregnancy and birth after transfer of embryos that developed from single-nucleated zygotes obtained by injection of round spermatids into oocytes. *Fertil Steril* 1998; **70**: 67–70.
[89] Al-Hasani S, Ludwig M, Palermo I *et al.* Intracytoplasmic injection of round and elongated spermatids from azoospermic patients: results and review. *Hum Reprod* 1999; **14**: 97–107.
[90] Ghazzawi IM, Alhasani S, Taher M, Souso S. Reproductive capacity of round spermatids compared with mature spermatozoa in a population of azoospermic men. *Hum Reprod* 1999; **14**: 736–740.
[91] Levran D, Nahum H, Farhi J, Weissman A. Poor outcome with round spermatid injection in azoospermic patients with maturation arrest. *Fertil Steril* 2000; **74**: 443–449.
[92] Vicdan K, Isik AZ, Delilbaşi L. Development of blastocyst-stage embryos after round spermatid injection in patients with complete spermiogenesis failure. *J Assist Reprod Genet* 2001; **18**: 78–86.
[93] Khalili MA, Aflatoonian A, Zavos PM. Intracytoplasmic injection using spermatids and subsequent pregnancies: round versus elongated spermatids. *J Assist Reprod Genet* 2002; **19**: 84–86.
[94] Sousa M, Cremades N, Silva J *et al.* Predictive value of testicular histology in secretory azoospermic subgroups and clinical outcome after microinjection of fresh and frozen–thawed sperm and spermatids. *Hum Reprod* 2002; **17**: 1800–1810.
[95] Saremi A, Esfandiari N, Salehi N, Saremi MR. The first successful pregnancy following injection of testicular round spermatid in Iran. *Arch Androl* 2002; **48**: 315–319.
[96] Amarin ZO, Jamal HS, Rouzi AA. Successful pregnancy after round spermatid microinjection. *Saudi Med J* 2002; **23**: 113–114.
[97] Ulug U, Bener F, Akman MA, Bahceci M. Partners of men with Klinefelter syndrome can benefit from assisted reproductive technologies. *Fertil Steril* 2003; **80**: 903–906.
[98] Benkhalifa M, Kahraman S, Biricik A *et al.* Cytogenetic abnormalities and the failure of development after round spermatid injections. *Fertil Steril* 2004; **81**: 1283–1288.
[99] Yanagida K, Yazawa H, Katayose H. Oocyte activation induced by spermatids and the spermatozoa. *Int J Androl* 2000; **23**: 63–65.
[100] Winston NJ, Braude PR, Johnson MH. Are failed-fertilized human oocytes useful? *Hum Reprod* 1993; **8**: 503–507.
[101] Sjögren A, Lundin K, Hamberger L. Intracytoplasmic sperm injection of 1 day old oocytes after fertilization failure. *Hum Reprod* 1995; **10**: 974–975.
[102] Chen C, Kattera S. Rescue ICSI of oocytes that failed to extrude the second polar body 6 h post-insemination in conventional IVF. *Hum Reprod* 2003; **18**: 2118–2121.
[103] Suganuma R, Walden CM, Butters TD *et al.* Alkylated imino sugars, reversible male infertility-inducing agents, do not affect the genetic integrity of male mouse germ cells during short-term treatment despite induction of sperm deformities. *Biol Reprod* 2005; **72**: 805–813.
[104] DeBaun MR, Niemitz EL, Feinberg AP. Association of in vitro fertilization with Beckwith–Wiedemann syndrome and epigenetic alterations of LIT1 and H19. *Am J Hum Genet* 2003; **72**: 156–160.

顕微授精

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はじめに

卵細胞質内精子注入法（intracytoplasmic sperm injection：ICSI）は体外受精で受精をはかることができない場合に適応となる媒精法の一法である．1992年にベルギーのグループがその成功を報じて以来，多くの重症男性因子例や受精障害例へ用いられてきた．安定した高い受精率が得られること，多精子受精の心配がないことが長所で，卵子損傷による変性が起こりうること，体外での操作による遺伝的影響の実態が不明なことが短所である．また，当面の課題としては，卵子と精子が存在するのに受精率が100％にならないことへの解決法を考案すること，良質な胚を作成するための良質な配偶子を選別する方法を考案することが挙げられる．以上のことをふまえて，ICSIの実践に役立つ重要なポイントを解説する．

マイクロマニピュレーション機器のセットアップ

詳細は成書に譲り，重要なポイントを述べる．マイクロインジェクターとマニピュレータのセットアップを行う．また，ICSIのための注入用ニードル（マイクロピペット）と卵保持用のホールディングピペットも取り付ける．機器をどのように調整するかは術者の好みが加わるところであるが，主役は配偶子ということを忘れてはならない．術者に都合がよいセットアップがベストではなく，卵子や精子にやさしいセットアップがベストである．例えば，インジェクターとチューブには，シリコンオイル，（古くは）フロリナート，蒸留水，空気などが充填されている．このなかで，空気を用いた場合ではそのメンテナンスが容易であるが，ニードルによるインジェクション操作（精子の吸引・排出など）は当然レスポンスが悪いので，トレーニングである程度は克服できると思うが，思わず大量の細胞質を吸引したり，大量の培養液を注入してしまうトラブルが発生しやすい．これらの操作上の問題はトレーニングにより解決できるかもしれないが，配偶子に傷害を与えるリスクをできる限り排除することを第一にするのであれば，多少面倒でもオイルや蒸留水をチュービングの充填剤として使うことが望ましいと思われる．

ICSIを実施した卵子の数ごとの受精障害発現率（すべての卵子が受精しなかった場合の出現頻度）を調べると，卵子数が少ないほど高く，卵子数が1個では20～25％が受精障害となることが判明している[1]．この原因として，セットアップ後，初めてのICSI操作ではセットアップのコンディションが十分でないことが考えられる．これからのARTでは採取する卵子数が減少するので，機器のコンディションを常にベストに保つことが要求される．

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