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POLYPS

Sessile polyps and pedunculated polyps respond differently to oral contraceptives

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Abstract
Endometrial polyp is the lesion frequently found by hysteroscopy. The presence of endometrial polyp is associated with abnormal uterine bleeding and is probably associated with infertility. Until today, clinical guidelines for endometrial polyp remain elusive. The aim of this preliminary study was to estimate whether the shape of endometrial polyps affects the response to the treatment with an oral contraceptive (OC). We performed a retrospective case series study on 50 women diagnosed as endometrial polyps by hysteroscopy and managed by the administration of OC. Hysteroscopy was performed in the follicular phase of the menstrual cycle before medical treatment. Endometrial polyps were classified as pedunculated polyps ($n = 25$) or sessile polyps ($n = 25$). After diagnosis, OC was administered for 2–5 months (median 3 months) intermittently: To quantify the regression rate of lesions, the area index of endometrial polyps was assessed. In the study group, when comparing the efficacy of treatment with OC, there was a statistically significant difference in the regression rate between sessile polyps and pedunculated polyps (76% *vs.* 44%, $p = 0.042$). We conclude that sessile polyps are more sensitive to OC treatment than pedunculated polyps, implying usefulness of the hysteroscopic classification of the shape of polyps in the management of endometrial polyps.

Keywords: *Endometrial polyps, oral contraceptive, pedunculated polyps, sessile polyps*

Introduction

Endometrial polyp is the lesion most frequently found by hysteroscopy [1]. The frequency is 10% in asymptomatic premenopausal women older than 30 [2]. The aetiology of endometrial polyp is proposed to be related to estrogenic stimulation [3], which is manifested by induction of polyps after the treatment with tamoxifen that provides an estrogenic stimulus to the endometrium [4]. Polyps can be histologically characterised as localised hyperplastic overgrowths of glands and stroma. In menstruating women, they may cause abnormal uterine bleeding, hypermenorrhea, and infertility. Hysteroscopic polypectomy has been shown to improve fertility rate, suggesting that endometrial polyp is an infertile factor [5–7]. In addition, endometrial polyp is recently suggested to be a risk factor for endometrial cancer and the presence of endometrial polyp should be carefully followed up to prevent disease progression [8].

Polyps can be surgically removed using curettage or hysteroscopy while some may regress on their own [9]. Although natural history of endometrial polyp is poorly understood, one study that followed seven asymptomatic women with endometrial polyps for 2.6 years demonstrated that both two women who had taken hormonal medications had regression of their polyps whereas two in five women who had not used any hormonal medications had spontaneous regression of their polyps [10].

We introduced an oral contraceptive (OC) for the treatment of endometrial polyp to confirm its expected effect to decrease the volume of endometrial polyp by suppressing endogenous estrogen. Conducting this treatment, we noticed that the response of polyps to OC appears to be different depending on the

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shape of polyp, i.e. pedunculated polyps and sessile polyps. Here we report the different regression rate by OC treatment between the two groups.

Materials and methods

We reviewed both electronic and paper medical records of all patients with endometrial polyps diagnosed at our institution between 1 January 2000 and 30 December 2008. The ultrasonographic evaluation with regard to the thickness of endometrium was assessed in all the patients. Indications for hysteroscopy were as follows, infertility screening, hypermenorrhea, abnormal uterine bleeding, and/or indicative findings for endometrial lesion (e.g. thick endometrial layer exceeding 15 mm). Prior to the hysteroscopy, endometrial cytology or biopsy was obtained and no malignancies were detected in all the subjects. In total, 212 women between 21- and 48-years old were diagnosed as endometrial polyp by hysteroscopy. Seventy-eight patients were treated by primary hysteroscopic resection of polyps and 46 patients were carefully observed without any interventions (Figure 1). These patients were randomly allocated to the treatments under informed choice of each patient. Of 88 patients that were treated by OC (Sophia A, a tablet containing norethisterone 1.00 mg and mestranol 0.05 mg, ASKA Pharmaceutical Co., Ltd., Tokyo, Japan) and were without hormonal medications before the administration of OC, 38 patients were excluded because of several reasons listed in the Figure 1 (reasons for dropout). The remaining 50 patients were subdivided into two groups according to the shape of polyps, i.e. pedunculated polyps or sessile polyps. No patients possessed both pedunculated and sessile polyps in this study. The patients took OC (1 tablet/day) for 21 days with subsequent withdrawal bleeding. The cycle of OC intake was between 2 and 5 (median 3.0). Follow-up included clinic visits and repeated hysteroscopy and transvaginal ultrasound examination including sonohysterography just after OC cycle in the follicular phase of the menstrual cycle. The measurement of polyps was done by transvaginal ultrasound. After defining the largest sagittal view of the lesion, the longest part of the lesion (A) and the orthogonalising part (B) was measured, and then A × B was calculated (Figure 2). The sum of A × B of all polyps was calculated in each patient and defined as an 'area index' of polyps to estimate the volume of these lesions. To evaluate the efficacy of treatment, the reduction percentage of this index more than 90% was defined as '*regression*' of the lesions. Less than 90% reduction was defined as the ineffective treatment. This report was exempt from the Institutional Review Board approval because the treatment required for these patients is a standard procedure for the treatment of endometrial polyps.

All procedures with regard to hysteroscopy were performed in the early follicular phase of the menstrual cycle on an outpatient basis. Each patient was discharged within 60 min. A 3.1-mm continuous-flow mechanical office hysteroscopy (Olympus, Tokyo, Japan) was inserted to the uterine cavity using atraumatic technique. Uterine cavity was distended with normal saline infusion. Uterine distension was achieved with a pressure cuff around the irrigation bag (25–35 mmHg). The cavity was systematically

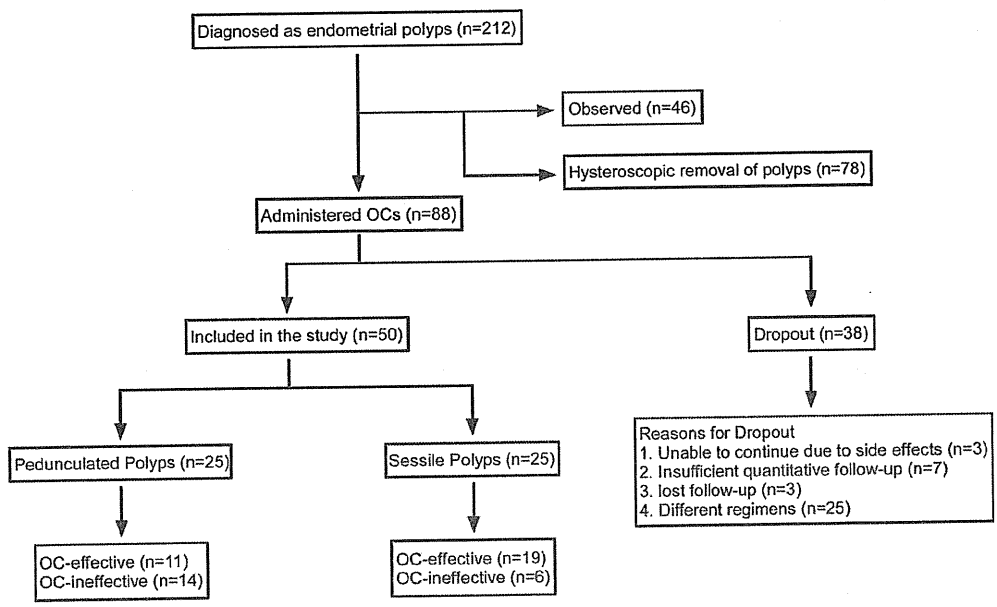

Figure 1. Flow chart of subjects in the study.

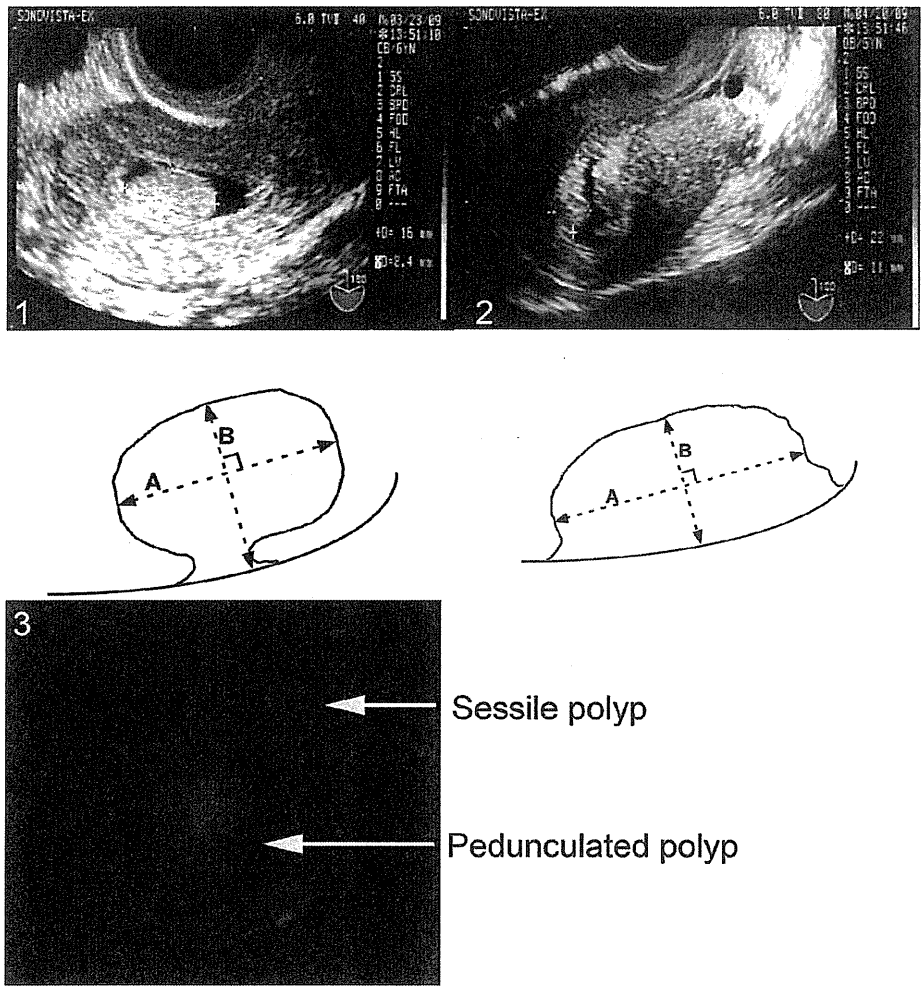

Figure 2. Representative size measurement and appearance of endometrial polyps. (1) Pedunculated polyps. (2) Sessile polyps. Schematic description of A and B is shown below. (3) Representative hysteroscopic appearance of polyps.

inspected for any abnormal findings. Good visualisation of the entire cavity with no structural abnormalities or hypervascularisation and the uniformly thin, homogenous-appearing endometrium without variations in thickness was considered normal. The typical appearance of pedunculated endometrial polyps was a well defined, homogenous, and smooth surface polypoid lesion attached to the uterine wall by a thick and short pedicle. These lesions were hemispheric, linguiform or coniform in shape. Even though the macroscopic appearance of sessile polyp was similar to that of the pedunculated ones, pedicle of sessile polyps was broad-based or flat as described in the previous report [11] and this atypical appearance of polyps was frequently observed. Most patients had no anesthesia during diagnostic hysteroscopy.

Statistical analysis was performed using Fisher's exact test for comparing the categoric variables between groups. The difference was analysed by Mann–Whittney's *U*-test for comparing continuous variables between groups. For all tests, a p value of less than 0.05 was considered to be statistically significant.

Results

The characteristics of the subjects are summarised in Table I. Sessile polyps were tended to be multiple and the number of lesions was higher in women with sessile polyps compared to those with pedunculated polyps (3.32 *vs.* 1.48, $p < 0.0001$). There were no statistically significant differences in the parity, age, and the frequency of abnormal uterine bleeding between the two groups. Patients with sessile polyps were significantly complicated by hypermenorrhea compared to those with pedunculated polyps (64% *vs.* 36%, $p = 0.022$, Table I).

After the OC treatment, the thickness of endometrium was decreased and the frequency of symptoms such as hypermenorrhea and abnormal uterine bleeding was also decreased in most of the patients. Hysteroscopic and sonographic findings after the

Table I. Patient demographic and treatment characteristics.

	Pedunculated polyp ($n=25$)	Sessile polyp ($n=25$)	p value
Age (year)	35.2 ± 7.1	32.8 ± 4.7	0.183*
Parity	0.32 ± 0.74	0.28 ± 0.61	0.899*
Abnormal uterine bleeding	36% (9/25)	40% (10/25)	>0.999†
Hypermenorrhea	28% (7/25)	64% (16/25)	0.022†
Number of lesions	1.48 ± 0.77	3.32 ± 1.84	<0.0001*
Area index of lesions (mm^2)	142.3 ± 89.9 (range 36.2–319.8)	125.7 ± 64.0 (range 18.59–285.0)	0.826*
Regression‡ by OC treatment	44% (11/25)	76% (19/25)	0.042†

*Mann–Whitney U test.
†Fisher's exact test.
‡The word '*regression*' denotes the reduction rate more than 90%.

treatment with OC revealed that the rate of regression was higher in the group of sessile polyps compared with the group of pedunculated polyps (76% *vs.* 44%, $p=0.042$, Figure 1 and Table I).

Discussion

The present study demonstrates that sessile polyps regress in a higher rate than pedunclated polyps under OC treatment. Several mechanisms can be speculated in OC-induced regression of the polyps. Apoptosis might be a mechanism in light of the finding that exposure to a monophasic OC for 30 days significantly increases endometrial apoptosis both in epithelial and stromal cells [12]. Another mechanism could be that the establishment of a steady estrogen–progestogen milieu induces endometrial quiescence, leading to the regression of endometrial polyps. Anti-inflammatory effects of progesterone might also be involved given that mast cell-associated inflammation is associated with endometrial polyp growth [13]. Nevertheless, it is difficult to speculate a specific reason why the sensitivity to OC is different between the two types of polyps. OC therapy is not without its own risks, particularly in an older (over 35-year old) population. Thus, it is debatable whether medical therapy is preferable. Furthermore, there is no longer-term follow-up as to how long the medical effects of the OCs will be maintained, even after cessation of this therapy. Given the average age of the populations studied, it is likely that the same endometrial environmental factors promoting the presence of the polyp(s) would still be extant at the completion of therapy. However, since there is no proper guideline for the treatment of endometrial polyp, it would be interesting to pursue the nature of endometrial polyp and therapeutic effect brought by OC.

Although the natural history of endometrial polyps is poorly known, one study demonstrated that smaller polyps regress with time and larger ones tend to persist [10]. The recent report speculated that polyps less than 10 mm may vanish [9]. In our study, as for pedunculated polyps, the size was tended to be small in OC-effective patients compared to OC-ineffective patients (113.23 *vs.* 168.96 mm^2, $p=0.123$) though the difference was not statistically significant. In contrast, sensitivity to OC treatment was unaffected by the size of polyps in patients with sessile polyps since the OC-effective patients possessed similar sized polyps compared with OC-ineffective patients (123.66 *vs.* 132.24 mm^2, $p=0.496$). These findings may suggest that the difference of cellular characteristics between the polyps is relevant to the overall regression rate and polyps more than 10 mm can regress by the OC treatment, at least in part.

An association between endometriosis and the presence of polyps has been suggested. A retrospective study found endometriosis in 27 of 32 (84.37%) women with polyps or polypoids at hysterosalpingography, compared with endometriosis in 19 of 88 (21.59%) patients without polyps or polypoids [14]. Because of the limited number of cases, we cannot address the relationship between the types of polyps and endometriosis in this study. However, it may be intriguing to study the relationship between endometriosis and the shape of polyps.

Some argues that polyps spontaneously regress [9]. And it may be the weakness of our study that we did not include comparison/control group of non-OC-treated patients. Nevertheless, our finding has an impact by demonstrating that one can predict the response to OC according to the shape of the polyps and hysteroscopy is useful to judge the shape of the polyps precisely. Although the detrimental complication rate associated with hysteroscopic surgery is low [15], gynecologists should remember complications brought by the hysteroscopic surgery such as intravasation of distension fluid, uterine perforation, infection, and haemorrhage. The fact that the shape of the polyps would be informative in selecting the treatment of endometrial polyps might be especially helpful for managing patients who prefer to avoid surgical treatment.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

1. Hinckley MD, Milki AA. 1000 office-based hysteroscopies prior to *in vitro* fertilization: feasibility and findings. Jsls 2004;8:103–107.
2. Clevenger-Hoeft M, Syrop CH, Stovall DW, Van Voorhis BJ. Sonohysterography in premenopausal women with and without abnormal bleeding. Obstet Gynecol 1999;94:516–520.
3. McGurgan P, Taylor LJ, Duffy SR, O'Donovan PJ. Are endometrial polyps from pre-menopausal women similar to post-menopausal women? An immunohistochemical comparison of endometrial polyps from pre- and post-menopausal women. Maturitas 2006;54:277–284.
4. Cohen I. Endometrial pathologies associated with postmenopausal tamoxifen treatment. Gynecol Oncol 2004;94:256–266.
5. Varasteh NN, Neuwirth RS, Levin B, Keltz MD. Pregnancy rates after hysteroscopic polypectomy and myomectomy in infertile women. Obstet Gynecol 1999;94:168–171.
6. Spiewankiewicz B, Stelmachow J, Sawicki W, Cendrowski K, Wypych P, Swiderska K. The effectiveness of hysteroscopic polypectomy in cases of female infertility. Clin Exp Obstet Gynecol 2003;30:23–25.
7. Stamatellos I, Apostolides A, Stamatopoulos P, Bontis J. Pregnancy rates after hysteroscopic polypectomy depending on the size or number of the polyps. Arch Gynecol Obstet 2008;277:395–399.
8. Bakour SH, Khan KS, Gupta JK. The risk of premalignant and malignant pathology in endometrial polyps. Acta Obstet Gynecol Scand 2002;81:182–183.
9. Haimov-Kochman R, Deri-Hasid R, Hamani Y, Voss E. The natural course of endometrial polyps: could they vanish when left untreated? Fertil Steril 2009;92:828.e11–828.e12.
10. DeWaay DJ, Syrop CH, Nygaard IE, Davis WA, Van Voorhis BJ. Natural history of uterine polyps and leiomyomata. Obstet Gynecol 2002;100:3–7.
11. Davis PC, O'Neill MJ, Yoder IC, Lee SI, Mueller PR. Sonohysterographic findings of endometrial and subendometrial conditions. Radiographics 2002;22:803–816.
12. Meresman GF, Auge L, Baranao RI, Lombardi E, Tesone M, Sueldo C. Oral contraceptives suppress cell proliferation and enhance apoptosis of eutopic endometrial tissue from patients with endometriosis. Fertil Steril 2002;77:1141–1147.
13. Al-Jefout M, Black K, Schulke L, Berbic M, Luscombe G, Tokushige N, Manconi F, Markham R, Fraser IS. Novel finding of high density of activated mast cells in endometrial polyps. Fertil Steril 2009;92:1104–1106.
14. McBean JH, Gibson M, Brumsted JR. The association of intrauterine filling defects on hysterosalpingogram with endometriosis. Fertil Steril 1996;66:522–526.
15. Williamson KM, Mushambi MC. Complications of hysteroscopic treatments of menorrhagia. Br J Anaesth 1996;77:305–308.

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Case of chronic ectopic pregnancy diagnosed in which the complete shape of the fetus was visible by ultrasonography

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Abstract

Preoperative diagnosis of chronic ectopic pregnancy is often difficult because of the high incidence of negative results on pregnancy tests as a consequence of the very small amount of live villi, subtle symptoms, and the poor specificity of ultrasonographic patterns. A 45-year-old woman was referred to our department for evaluation of a mass 8 cm in diameter with solid parts in the right adnexal area. Transvaginal ultrasonography showed a mass consisting of a cystic part with an irregular thick capsule distinct from the right ovary. In the center of the cystic part, a fetus-like image, 20 mm in length was seen. Preoperative diagnosis was confirmed by the laparoscopy, which revealed a swollen right tube containing a fetus with highly necrotic changes. This case was unique because chronic ectopic pregnancy was detected at an early stage before absorption of the conceptus occurred, which coincidentally is an appropriate time for morphological diagnosis.

Key words: chronic ectopic pregnancy, human chorionic gonadotropin, laparoscopy, magnetic resonance imaging, ultrasonography.

Case Report

A 45-year-old woman, gravida 4, para 2, presented to her primary gynecologist's clinic with complaints of lower abdominal distention for one month. She had no gastrointestinal symptoms, her appetite was normal and her bodyweight had not changed. Transvaginal ultrasonography revealed a mass, 8 cm in diameter, in the right adnexal area, similar to an ovarian neoplasm, and she was referred to our department. On gynecological examination, a fist-sized, immobile, tender, elastic hard mass was detected in the right side of the cul-de-sac, and no abnormal finding was shown in the left adnexal area. Transvaginal ultrasonography revealed that this mass consisted of a cystic part with an irregular thick capsule distinct from the right ovary (Fig. 1a). There was no abnormal finding in the uterus or the left ovary, and free fluid was not observed by transvaginal ultrasonography. In the center of the cystic part, a fetus-like image, 20 mm in length, was seen. Magnetic resonance imaging (MRI) showed the lesion with central necrosis and a thick fibrous capsule (Fig. 1b). Tests for both human chorionic gonadotropin (hCG) and β-hCG were negative. The plasma α-fetoprotein (αFP) level (normal; 0–9) was elevated to 116 ng/mL. The plasma CA19-9 and CA125 levels were 17 U/mL and 13 U/mL, respectively. Hereafter, to clarify the clinical time course, we define the day of her first visit to our hospital as day 0. For example, one month before and 10 days after her first visit are

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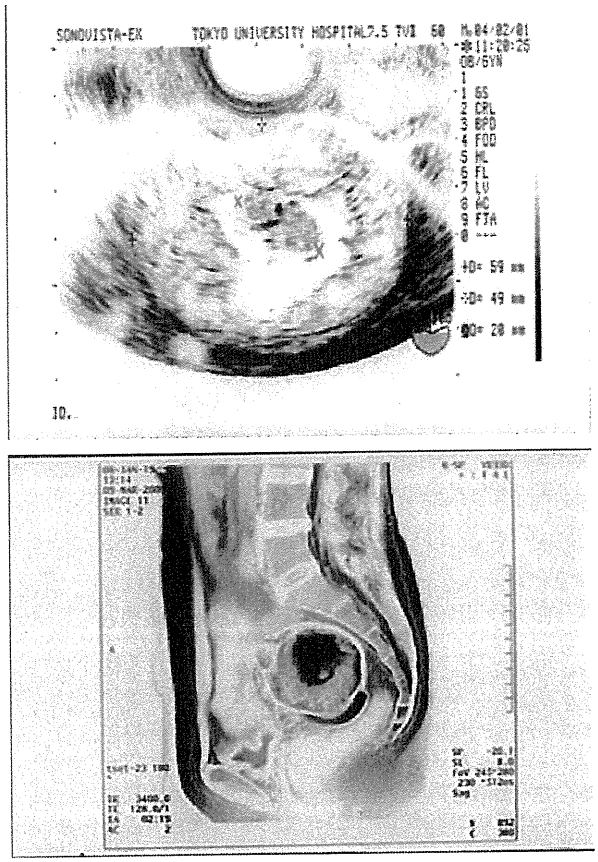

Figure 1 (a) Transvaginal ultrasonography revealed a right adnexal cystic mass with irregular thick capsule containing a fetus-like image. (b) Magnetic resonance imaging, T2-weighted. αFP, α-fetoprotein.

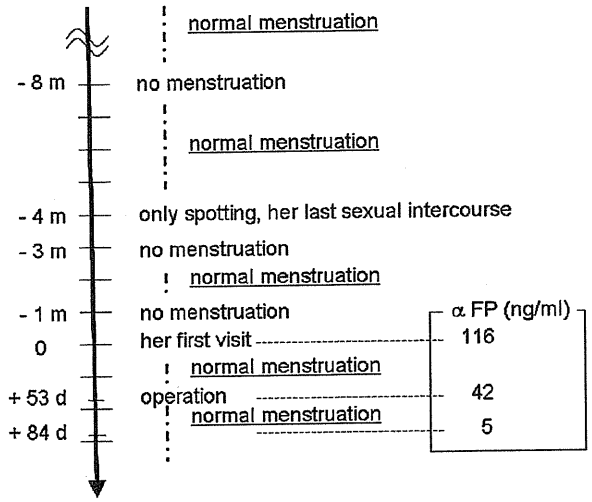

Figure 2 The clinical time course. The vertical line indicates the time course. d, day; m, month.

Figure 3 Laparoscopic view. The ampulla of the right tube enlarged.

mentioned as –1 month (m) and +10 days (d), respectively. The clinical time course is indicated in Figure 2. Although the patient had a regular menstrual cycle of 28 days before –9 m, she had no menstruation in –8 m, –4 m (only spotting was observed), –3 m, and –1 m. The last sexual intercourse before consultation was during –4 m. On +52 d, the plasma αFP level dropped to 42 ng/mL and concomitantly, the size of the mass decreased to 5.5 cm in diameter, although no change was found in the shape of the fetus-like image. Taking these findings into consideration, we diagnosed the mass as a chronic ectopic pregnancy; however, we still couldn't exclude the possibility of an ovarian neoplasm with just these serological tests and images, in spite of the decrease in its size and the plasma αFP level within 2 months. In this context, with a thorough informed consent, we performed laparoscopy on +53 d in order to make a pathological diagnosis with a minimal invasive surgery. A 6-cm mass was noted in the ampullary portion of the right tube (Fig. 3). Filmy adhesion between the mass and the right ovary, surrounding mesenterium, and the posterior broad ligament was found. The excised right tube is shown in Figure 4a. The mass was filled with brownish fluid, containing a fetus with highly necrotic changes (Fig. 4b). The crown–rump length of the fetus was about 2.0 cm. Histological evaluation confirmed a diagnosis of chronic right tubal pregnancy. The plasma αFP level gradually decreased thereafter, reaching normal limits one month after surgery.

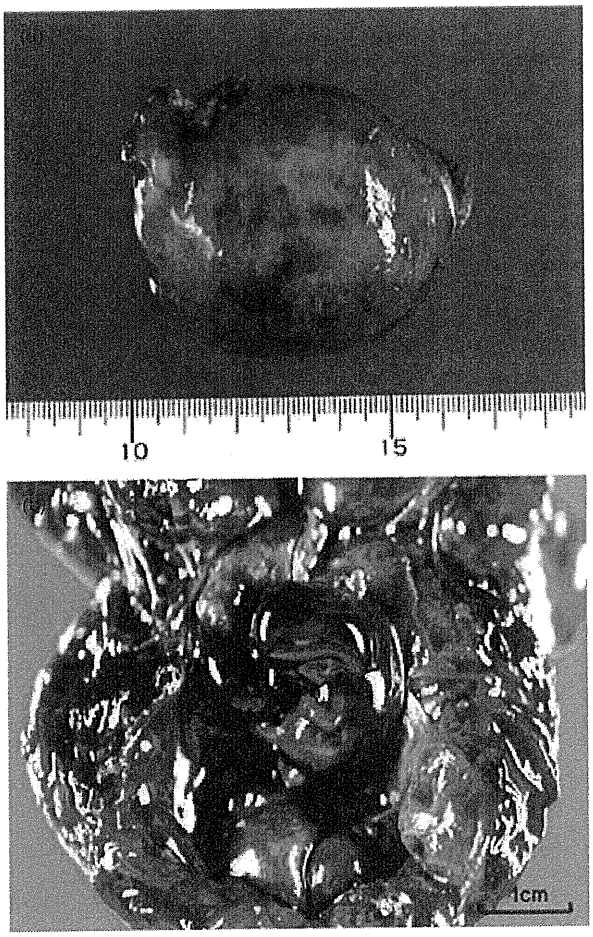

Figure 4 Macroscopic views of the excised right tube. (a) As a whole. (b) Inside the mass, a fetus of about 9 weeks of gestational age was found.

Discussion

This is the first case report of chronic ectopic pregnancy in which the complete shape of the fetus was visible. Chronic ectopic pregnancy is a tubal gestation that has undergone abortion or repeated minor bleeding episodes, in which the hemodynamic insult is subclinical and self-limiting.[1] Preoperative diagnosis is often difficult because of the high incidence of negative pregnancy tests as a result of a very small amount of live villi, subtle symptoms, and the poor specificity of ultrasonographic patterns.[2] Because hCG levels cannot reliably eliminate the risk of tubal rapture in the case of chronic ectopic pregnancy, it should be considered in the differential diagnosis of patients with an adnexal mass even with low hCG levels and regular menses.[3–5] Curry *et al.* reported the case of chronic ectopic pregnancy, diagnosed using hysterosalpingography, as a rare case.[6] It was reported that an adnexal mass of chronic ectopic pregnancy, which was adherent to omentum, was demonstrated with extensive external vascularization, but with no internal blood flow on Doppler ultrasonography.[7] In our case, there was only filmy adhesion around the right adnexal mass, indicating that Doppler ultrasonography might not give us useful information for diagnosis. The mass that occurs as the final form of chronic ectopic pregnancy is usually a conglomeration produced by adhesion between the inflamed tube after degeneration of the conceptus and surrounding structures, often containing blood and necrotic debris.[8,9] In most cases, it occupies one adnexa and the cul-de-sac, yielding the heterogenous echo pattern. Some cases, around 10% of the cases Turan *et al.* examined, revealed a predominantly solid pattern.[8] Another ultrasonographic finding that may help diagnosis is simple fluid collection in the pelvic cavity resulting from old blood, although a big difference in its incidence has been seen depending on the report. In summary, the sonographic pattern of ectopic pregnancy is very similar to that of pelvic inflammatory diseases and ovarian neoplasms without any specific feature. That is why there are few papers reporting that ultrasound plays a key role in its diagnosis. Accordingly, the differentiation of chronic ectopic pregnancy without positive pregnancy tests from those other pelvic pathologies can only rely on a history of amenorrhea. Consequently, in almost all cases, diagnosis is possible only after pathological examination. In our case, however, the mass consisted only of the conceptus and a thick capsule, accompanied by infiltration of blood cells, fibrin deposition, and fibrotic change. We speculate that, if the natural course had been observed, it would have resulted in findings identical to those of other cases reported previously. This case was intriguing and novel because chronic ectopic pregnancy was detected at an early stage before absorption of the conceptus occurred, which coincidentally is an appropriate time for morphological diagnosis.

References

1. Cole T, Corlett R. Chronic ectopic pregnancy. *Obstet Gynecol* 1982; 59: 63–68.
2. Ugur M, Turan C, Vicdan K, Ekici E, Oguz O, Gokman O. Chronic ectopic pregnancy: A clinical analysis of 62 cases. *Aust N Z J Obstet Gynecol* 1996; 36: 186–189.

3. Brennan DF, Kwatra S, Kelly M, Dunn M. Chronic ectopic pregnancy – two cases of acute rupture despite negative beta hCG. *J Emerg Med* 2000; **19**: 249–254.
4. Porpora MG, Alò PL, Cosmi EV. Unsuspected chronic ectopic pregnancy in a patient with chronic pelvic pain. *Int J Gynaecol Obstet* 1999; **64**: 187–188.
5. Dunn RC, Taskin O. Chronic ectopic pregnancy after clinically successful methotrexate treatment of ectopic pregnancy. *Int J Gynaecol Obstet* 1995; **51**: 247–249.
6. Curry NS, Blackwood GA, Tsai CC. Diagnosis of chronic ectopic gestation by hysterosalpingography. *Abdom Imaging* 1999; **24**: 98–99.
7. Abramov Y, Nadjari M, Shushan A, Prus D, Anteby SO. Doppler findings in chronic ectopic pregnancy: Case report. *Ultrasound Obstet Gynecol* 1997; **9**: 344–346.
8. Turan C, Ugur M, Dogan M, Ekici E, Vicdan K, Gokman O. Transvaginal sonographic findings of chronic ectopic pregnancy. *Eur J Obstet Gynecol Reprod Biol* 1996; **67**: 115–119.
9. Di Spiezio Sardo DI, Mastrogamvrakis A, Taylor G, Sharma M, Buck L, Magos A. Chronic ectopic pregnancy diagnosed incidentally in an infertile woman: A case report. *J Reprod Med* 2004; **49**: 992–996.

Defining Cell Identity by Comprehensive Gene Expression Profiling

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Abstract: The human body is composed of 60 trillion cells, which have their origin in a fertilized egg. During development, the potential of a cell or tissue can be achieved by environmental manipulation. Then, what molecular determinants underlie or accompany the potential of the cells? To obtain a broader understanding of these problems, it is important to analyze all transcripts / genes in a wide selection of cell types. The development of microarray technologies, which allow us to undertake parallel analyses of many genes, has led to a new era in medical science. In this review, we show that the global expression data have clearly elucidated discernible major trends of the phenomenon in preimplantation development and epithelial-mesenchymal transition, and of the character of marrow stromal cells, which are attracting a great deal of attention as they represent a valuable source of cells for regenerative medicine. One of the interesting results is obtained from microarray data of marrow stromal cells: OP9 cells that have been recognized as a type of niche-constituting preadipocyte derived from marrow stroma, are found to be chondroblasts. We also describe what effect each type of expression data would bring to reproductive and regenerative medicine, as well as offering an excellent model of cell differentiation in biology.

Keywords: Gene chip array, cell potency, early embryogenesis, transdifferentiation, cellular plasticity, hyaline cartilage formation, endochondral ossification, bioinformatics.

INTRODUCTION

What type of "human" do you like? – do you judge by his appearance and/or his character? When the word "human" is replaced by "cell" in this question, the present situation surrounding regenerative medicine emerges. With the derivation of pluripotent embryonic stem (ES) and somatic stem cells that can differentiate into many different cell types, excitement has increased for the prospect of replacing dysfunctional or failing cells and organs. Somatic stem cells have been identified in hematopoietic [1], hepatic [2], epidermal [3], gastrointestinal [4], neural [5, 6], muscle [7], and bone marrow [6-8] tissues. Many researchers have since demonstrated the developmental pluripotency of these cells. What is not yet clear, however, are the critical molecular mechanisms that can harness or manipulate the potential of cells to foster therapeutic applications targeted to specific tissues.

Then, what are the appearance and the character of these cells? This question is: can the diverse morphology of the cell and/or the differential activities of genes provide the distinction between totipotent cells, pluripotent cells and terminally differentiated cells? One approach to this question is through markers that appear on the surfaces of cells by flow cytometric analysis. Hematopoietic stem cells (HSCs) are somatic stem cells found in the bone marrow and the precursor cells that give rise to all the types of both the myeloid and lymphoid lineages. This includes monocytes and macrophages, neutrophils, basophils, eosinophils, T cells, B cells, NK cells, erythrocytes, megakaryocytes, and dendritic cells. How do researchers find the desired cell populations and stem cells at a specific hierarchical stage? Multipotent HSCs present various clusters of differentiation markers on their surface: CD34, CD38, CD90, CD133, Lin, Thy1, and CD45. Understanding the cell lineage of HSCs will eventually allow the generation of expanded populations of HSCs *ex vivo* that can be used therapeutically.

Another powerful approach to these questions is that of systematic genomic methodologies [9]. One of these methods, cDNA microarray/chip technology, is providing useful information [10-13]. Because of the logical connection between the function of a gene and its pattern of expression, the correlation of gene expression patterns with the variation in the phenotype of the cell can begin the process by which the function of a gene can be inferred. Similarly, the patterns of expression of known genes can reveal novel phenotypic aspects of the cell and tissues studied [14-16]. In this review we describe the use of microarray technology to determine cell identity based on gene expression pattern, with applications in regenerative medicine, especially preimplantation embryos, epithelial-mesenchymal transition and the mesenchymal stem cells.

THE BIG WAVE IN PREIMPLANTATION EMBRYO DEVELOPMENT

Preimplantation development encompasses the period from fertilization to implantation, and is marked by a number of critical sequential events. Understanding preimplantation development is important both for basic reproductive biology and for practical applications, including regenerative medicine and stock breeding. Preimplantation development is marked by 4 major events: the transition of maternal transcripts to zygotic transcripts, compaction, the first lineage differentiation into inner cell mass (ICM) and trophectoderm (TE), and implantation. The scarcity of the materials of preimplantation embryos, both in size (diameter <100 um) and in quantity (only a few to tens of oocytes from each ovulation), has hampered molecular analysis of preimplantation

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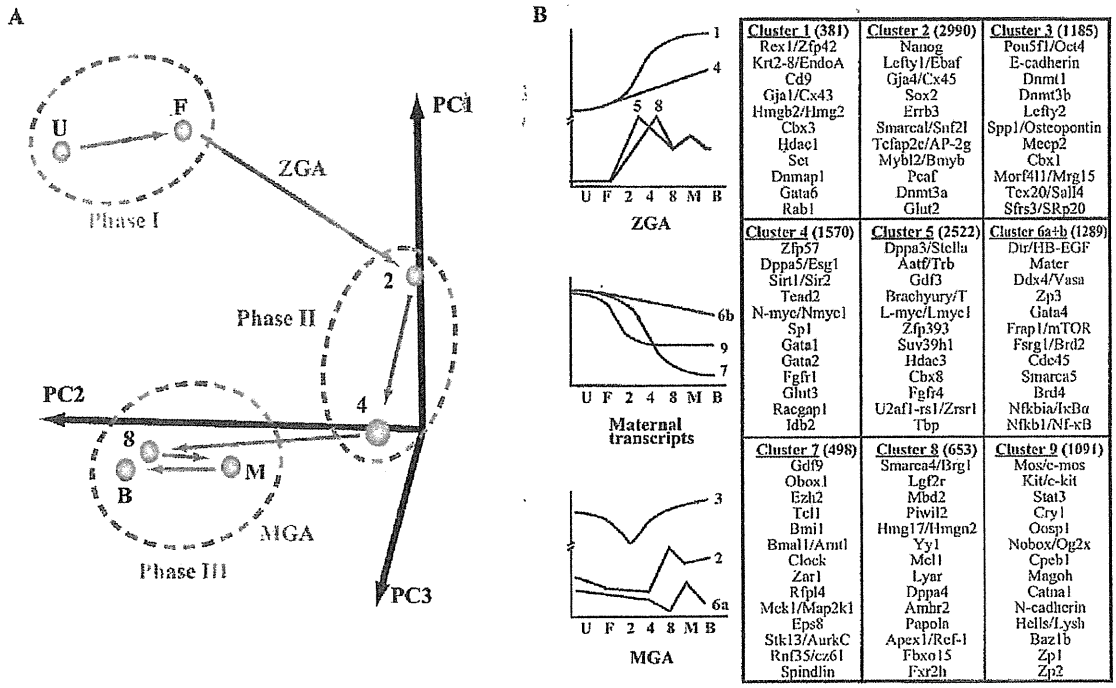

Cluster 1 (381)	Cluster 2 (2990)	Cluster 3 (1185)
Rex1/Zfp42	Nanog	Pou5f1/Oct4
Krt2-8/EndoA	Lefty1/Ebaf	E-cadherin
Cd9	Gja4/Cx45	Dnmt1
Gja1/Cx43	Sox2	Dnmt3b
Hmgb2/Hmg2	Errb3	Lefty2
Cbx3	Smarcal/Snf2l	Spp1/Osteopontin
Hdac1	Tcfap2c/AP-2g	Mecp2
Set	Mybl2/Bmyb	Cbx1
Dnmap1	Pcaf	Morf4l1/Mrg15
Gata6	Dnmt3a	Tex20/Sall4
Rab1	Glut2	Sfrs3/SRp20
Cluster 4 (1570)	**Cluster 5 (2522)**	**Cluster 6a+b (1289)**
Zfp57	Dppa3/Stella	Dtr/HB-EGF
Dppa5/Esg1	Aatf/Trb	Mater
Sirt1/Sir2	Gdf3	Ddx4/Vasa
Tead2	Brachyury/T	Zp3
N-myc/Nmyc1	L-myc/Lmyc1	Gata4
Sp1	Zfp393	Frap1/mTOR
Gata1	Suv39h1	Fsrg1/Brd2
Gata2	Hdac3	Cdc45
Fgfr1	Cbx8	Smarca5
Glut3	Fgfr4	Brd4
Racgap1	U2af1-rs1/Zrsr1	Nfkbia/IκBα
Idb2	Tbp	Nfkb1/Nf-κB
Cluster 7 (498)	**Cluster 8 (653)**	**Cluster 9 (1091)**
Gdf9	Smarca4/Brg1	Mos/c-mos
Obox1	Lgf2r	Kit/c-kit
Ezh2	Mbd2	Stat3
Tcl1	Piwil2	Cry1
Bmi1	Hmg17/Hmgn2	Oosp1
Bmal1/Arntl	Yy1	Nobox/Og2x
Clock	Mcl1	Cpeb1
Zar1	Lyar	Magoh
Rfpl4	Dppa4	Catna1
Mek1/Map2k1	Amhr2	N-cadherin
Eps8	Papoln	Hells/Lsh
Stk13/AurkC	Apex1/Ref-1	Baz1b
Rnf35/cz61	Fbxo15	Zp1
Spindlin	Fxr2h	Zp2

Fig. (1). Expression Profiling of Preimplantation Embryos.

A. Principal component analysis based on the expression data by gene chip array.

A matrix of scatter plots. U, F, 2, 4, 8, M, and B denote unfertilized egg, fertilized egg, 2-cell embryo, 4-cell embryo, 8-cell embryo, morula, and blastocyst, respectively. Each scatter plot shows the comparison of gene expression between embryo stages.

B. Expression changes of individual genes analyzed by the k-means non-hierarchical clustering method. Gene expression patterns can be assigned to three main groups. Group 1 (Cluster 1, 4, 5 and 8) appears to represent ZGA genes that are first activated from the zygotic genome. Group 2 (Cluster 7 and 9) represents maternal transcripts with distinctive patterns of degradation during preimplantation development. Group 3 (Cluster 2 and 3) appears to represent genes that follow a combination of these two patterns.

(Modified from Hamatani *et al.* Dev Cell, 2004, 6, 117 [18]).

embryos. Recent progress in RNA amplification methods using *in vitro* transcription and microarray platforms, including genes unique to preimplantation embryos, allows us to apply global gene expression profiling to the study of preimplantation embryos [17]. Hamatani *et al.* reported, for the first time, the global gene expression profiles of preimplantation embryos at all stages [18]. More than half of 21,939 gene features show statistically significant changes during preimplantation development. Pair-wise comparison, hierarchical clustering analysis, and principal component analysis (PCA) reveal two major transient waves of de novo transcription as follows: the first wave corresponds to zygotic genome activation (ZGA); the second wave, mid-preimplantation gene activation (MGA), contributes dramatic morphological changes during late preimplantation development (Fig. (**1A**)). Unsupervised methods such as principal component analysis (PCA) can transform the original features into new features (principal components (PC)), each PC representing a linear combination of the original features. PCA reduces input dimensionality by providing a subset of components that captures most of the information in the original data. For example, those genes that are highly correlated with the most informative PCs could be selected as classifier inputs, rather than a large dimension of original variables containing redundant features. To trace the expression changes of individual genes, statistically significant genes are analyzed by the k-means non-hierarchical clustering method. Gene expression patterns of these clusters can be assigned to three main groups (Fig. (**1B**)). The first group appears to represent ZGA genes that are first activated from the zygotic genome (Fig. (**1B**) Clusters 1, 4, 5, and 8). According to Gene Ontology (GO) terms [19] by MAPPFinder [20, 21], ZGA is suggested not to be promiscuous and to contribute mainly to the preparation of basic cellular machinery during the 2-cell and the 4-cell stages. The second group represents maternal transcripts with distinctive patterns of degradation during preimplantation development (Fig. (**1B**) Clusters 7 and 9). The third group appears to represent genes that follow a combination of these two patterns (Fig. (**1B**) Clusters 2 and 3). Genes whose expression first significantly increases from the 4-cell to 8-cell stage are identified as MGA genes, of which there are 4,216. The functional assignment of these genes by MAPPFinder characterizes the function of the MGA genes by the following three representative GO terms: "endopeptidase inhibitor," "intercellular junction," and "DNA (cytosine-5-)-methyltransferase." The implication of these GO terms and the timing of MGA seems consistent with the proposed role of MGA in compaction, cavitation, and the first differentiation of ICM and TE. Expression profiling of embryos treated

with inhibitors of transcription and translation reveals that the translation of maternal RNAs is required for the initiation of ZGA, suggesting a cascade of gene activation from maternal RNA/protein sets to ZGA gene sets and thence to MGA gene sets.

Decreasing oocyte competence with maternal aging is a major concern in human infertility because the rate of late childbearing is increasing even though reproductive capacity in women declines dramatically with advancing age. Studies of molecular mechanisms involved in the decline of oocyte quality with maternal age could have important implications for the efficacy and safety of clinical ooplasmic donation to rejuvenate aging oocytes. Hamatani *et al.* and Pan *et al.* also reported age-associated alteration of gene expression patterns in mouse oocytes, which has implications for aging research [22, 23]. Genes related to oxidative stress (e.g., Sod1, Apacd and Txn1), mitochondrial function (e.g., Sdha, Pdhb and Cyb5), chromatin structure (e.g., Hdac2, Hmgb3 and Bmi1), DNA methylation (e.g., Dnmt1, Dnmt3b, and Dnmt3L), and genome stability (e.g., Tert, Exo1, and Msh3) are altered with aging. Furthermore, kinetochore components of the spindle assembly checkpoint (e.g., Bub1, BubR1, Aurora kinase) and Cdc20, a critical activator of the Anaphase Promoting Complex, may contribute to aneuploidy in aged oocytes [23].

These comprehensive expression profiles of the majority of genes should give a baseline for analysis of the complex gene regulatory networks in normal mouse preimplantation and for comparative analysis for other mammalian species, including humans.

WHAT'S GOING ON IN AN EPITHELIAL-MESENCHYMAL TRANSITION (EMT)?

The conversion of an epithelial cell to a mesenchymal cell is critical to vertebrate embryogenesis and a defining structural feature of organ development, such as forming fibroblasts in injured tissues [24, 25], or in initiating metastases in epithelial cancer [26-29]. From a general perspective, EMT is about disaggregating epithelial units and reshaping epithelia for movement. Epithelia in transition lose polarity, tight junctions, adherens junctions, desmosomes and cytokeratin intermediate filaments in order to rearrange their F-actin stress fibers and express lamellopodia.and filopodia. This phenotypic conversion requires the molecular reprogramming of epithelia with new biochemical instructions. It is known that commonly used molecular markers for EMT include increased expression of N-cadherin and vimentin, nuclear localization of beta-catenin, and increased production of the transcription factors such as Snail, Twist, and SIP1/ZEB2. Much of this conversion, however, has been studied during experiments that expose new transduction and signaling pathways in epithelia, and more recently in fibrogenic tissues. It is not yet clear whether the fibroblast transition of EMT is an expected middle phase of transdifferentiating epithelia, or whether EMT producing fibroblasts is an arrested form of transdifferentiation.

EMT is easily engaged by a combination of cytokines associated with proteolytic digestion of basement membranes upon which epithelia reside. We analyzed PCA and hierarchical clustering method of the gene expression pattern of the renal tubular cells and mammary gland cells. If PC1 were used to identify genes that are differentially expressed between phenotypes, then genes that are strongly associated with PC1 would be selected. If both PC axes are used, then genes strongly associated with two groups would be selected. We then identified the genes which discriminate between the renal tubular and the mammary gland epithelial cells (PC1), or EMT-induced and non-induced cells (PC3) (Fig. (2)). Undergoing EMT identifies the genes that discriminate between the renal tubular and the mammary gland epithelial cells (PC1), or EMT-induced and non-induced cells (PC3) (Table 1).

The advanced study of the genes identified by PCA would yield new insight regarding EMT, and achieve a breakthrough in understanding the molecular mechanisms of

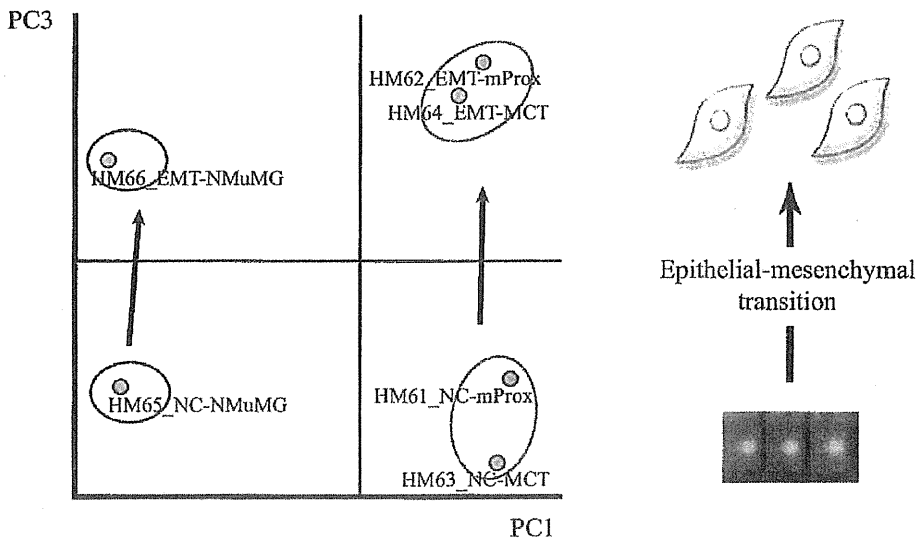

Fig. (2). EMT related genes by gene chip analysis.

Principal component analysis based on the gene expression pattern of the renal tubular cells and mammary gland cells.

Table 1. List of Genes that Had Up-Regulated (Positive) and Down-Regulated (Negative) Expression Related to EMT (PC3 Axis)

Positive			Negative		
Gene Symbol	**Gene Title**	**Representative Public ID**	**Gene Symbol**	**Gene Title**	**Representative Public ID**
Ccng2	cyclin G2	U95826	Bach1	BTB and CNC homology 1	NM_007520
Ccni	cyclin I	NM_017367	Cdc42bpa	Cdc42 binding protein kinase alpha	BM117074
Ctgf	connective tissue growth factor	NM_010217	Dnm1	dynamin 1	L31397
Dock7	Dedicator of cytokinesis 7	BB463580	Foxb1	forkhead box B1	U90538
Dok1	docking protein 1	BC006868	Gprc5c	G protein-coupled receptor, family C, group 5, member C	BC008228
Fgfr1	Fibroblast growth factor receptor 1	M33760	Il13ra1	interleukin 13 receptor, alpha 1	S80963
Gja1	gap junction membrane channel protein alpha 1	BB039269	Kcnk5	potassium channel, subfamily K, member 5	AF319542
Gtpbp4	GTP binding protein 4	AI987834	Kif13a	kinesin family member 13A	AB037923
Hgfac	hepatocyte growth factor activator	NM_019447	Kif17	kinesin family member 17	AW492270
Hoxa3	homeo box A3	BB496114	Mark2	MAP/microtubule affinity-regulating kinase 2	B1686265
Hoxb8 /b7	homeo box B8 / homeo box B7	X13721	Mef2d	myocyte enhancer factor 2D	NM_133665
Il15ra	interleukin 15 receptor, alpha chain	NM_008358	Mrpl51	mitochondrial ribosomal protein L51	AI594880
Irx2	Iroquois related homeobox 2	AF295369	Mxd4	Max dimerization protein 4	BE291523
Itga5	integrin alpha 5	BB493533	Neu1	neuraminidase 1	AI649303
Itgb1	integrin beta 1	BM120341	Rel	reticuloendotheliosis oncogene	NM_009044
Mapkbp 1	Mitogen activated protein kinase binding protein 1	BQ174980	Rgnef	Rho-guanine nucleotide exchange factor	BG069493
Mdm2	transformed mouse 3T3 cell double minute 2	X58876	Rps6kb2	ribosomal protein S6 kinase, polypeptide 2	NM_021485
Ncam1	neural cell adhesion molecule 1	NM_010875	Slc24a1	solute carrier family 24, member 1	BC016094
Pdgfa	platelet derived growth factor, alpha	BB371842	Slc25a19	solute carrier family 25, member 19	AV338420
Prkcc	protein kinase C, gamma	NM_011102	Slc25a22	solute carrier family 25 , member 22	AK018760
Rab23	RAB23, member RAS oncogene family	NM_008999	Slc40a1	solute carrier family 40 , member 1	AF226613
Rasa3	RAS p21 protein activator 3	NM_009025	Stat1	signal transducer and activator of transcription 1	AW214029
Rb1	retinoblastoma 1	NM_009029	Tgfa	transforming growth factor alpha	M92420
Sbno1	Sno, strawberry notch homolog 1	BC023136	Ubp1	upstream binding protein 1	NM_013699
Slc1a4	solute carrier family 1, member 4	BB277461	Usp12	ubiquitin specific protease 12	AF441835
Slc34a1	solute carrier family 34 , member 1	AI788646			
Slc4a7	Solute carrier family 4, member 7	AW555750			
Slc7a2	solute carrier family 7, member 2	M62838			
Ube1y1	ubiquitin-activating enzyme E1, Chr Y 1	X62581			
Vegfa	vascular endothelial growth factor A	NM_009505			
Wnt6	wingless-related MMTV integration site 6	NM_009526			
Wnt7b	wingless-related MMTV integration site 7B	W29605			

drug delivery for specific anti-cancer drugs, especially those affecting metastasis. Progress in understanding EMT has been an exercise in coming to appreciate the level of complexity required for changing cellular identity. The mecha-

nism of EMT highlights an integration of nuclear regulation and network signaling with alterations in the microenvironment to create a moving cell; in this sense, basic concepts based on EMT mechanisms would thus hold great promise for regenerative medicine.

GET OP9 CELLS DEAD TO RIGHTS

The concept of regenerative medicine refers to the cell-mediated restoration of damaged or diseased tissue. Candidate cell sources for tissue regeneration include ES cells, fetal cells, and adult cells such as marrow stromal cells, each of which has both advantages and drawbacks. Clinical trials with marrow stromal cells have been performed in patients with osteogenesis imperfecta and osteoporosis, and marrow stromal cells are expected to be a good source of cell therapy.

Bone marrow-derived stem cells can be transdifferentiated into multilineage cells, such as muscle [30] from mesoderm, lung [31] and liver [31, 32] from endoderm, and brain [33-36] and skin [31] from ectoderm. Somatic stem cells are more desirable than ES cells for cell therapeutics because of ethical considerations and the possible immunologic rejection of ES cells. Mesenchymal stem cells have become the most popular somatic stem cells in medicine and biology, not least because of their high reproductive capability *in vitro*.

Chondrocytes differentiate from mesenchymal cells during embryonic development [37], and the phenotype of the differentiated chondrocyte is characterized by the synthesis, deposition, and maintenance of cartilage-specific extracellular matrix molecules, including type II collagen and aggrecan [38-40]. The phenotype of differentiated chondrocytes is rapidly lost since it is unstable in culture [41-44]. This process is referred to as 'dedifferentiation' and is a major impediment to the use of mass cell populations for therapy or tissue engineering of damaged cartilage. When isolated chondrocytes are cultured in a monolayer at low density, the typical round chondrocytes morphologically transform into flattened fibroblast-like cells, with profound changes in biochemical and genetic characteristics, including reduced synthesis of type II collagen and cartilage proteins [45].

We established several stromal cells from murine bone marrow cultures [46]. One of them, KUSA-A1 cells, displays osteogenetic characteristics *in vitro* and *in vivo*. In order to clarify the specific gene expression profile of KUSA-A1, other established stromal cells, KUM5, 9-15c, KUSA-O, H-1/A [47], and mouse embryonic fibroblasts, we compared the expression levels of approximately 23,000 genes by using the Affymetrix gene chip oligonucleotide arrays. Of the 23,000 genes represented on the gene chip, chondrocyte-specific or -associated genes such as type II collagen α1, type XI collagen α1, Sox9, proline arginine-rich end leucine-rich repeat, and cartilage oligomeric matrix protein are more strongly expressed in KUM5 cells than in other marrow-derived mesenchymal cells. Does a gene expression pattern reflect the character of the cells *in vitro* and/or *in vivo*? – The answer is yes: KUM5 cells generate hyaline cartilage and exhibit endochondral ossification *in vitro* (Fig. (**3B**)) and *in vivo* [48].

Surprisingly, OP9 cells [49] also express these chondrocyte-specific or -associated genes at higher levels: the type II collagen α1, and type XI collagen α1 genes are expressed in OP9 cells at more than 10-fold higher levels than in 9-15c, KUSA-O, H-1/A, primary embryonic fibroblasts, or even KUM5 chondroblasts. In addition, expression of 'structural proteins' on Gene Ontology, including the extracellular matrix, is much higher by OP9 and KUM5 cells than by non-chondrogenic cells such as KUSA-A1, H-1/A, and 9-15c, implying that the OP9 and KUM5 cells are mainly engaged in synthesizing extracellular matrix. We also performed hierarchical clustering and PCA, based on the microarray data (Fig. (**3A**)). KUM5 and OP9 cells are grouped into the same subcategory and can clearly be separated from other stromal cells based on the expression data of cell surface markers and cell-type-specific genes, implying that KUM5 and OP9 cells have chondrogenic potential.

Are OP9 cells chondroblasts *in vitro* and/or *in vivo*? - the answer, again, is yes; OP9 cells are induced into the chondrogenic lineage by the pellet culture method (Fig. (**3C**)), and the OP9 pellets (micromasses) implanted in mice form the type II collagen-positive hyaline cartilage [48]. OP9 cells are derived from macrophage colony-stimulating factor-deficient osteopetrotic mice, and have also been used as feeder cells for embryonic stem cells [50-52]. The cells identified as a key participant in regulating the number of adult stem cells or hematopoietic stem cells are now considered to be of an osteoblastic lineage [53, 54]. OP9 cells have been recognized as a niche-constituting preadipocyte; however their true face is a chondroblast. We have two different types of cells, osteoblasts (KUSA-A1) and chondroblasts (OP9 and KUM5), showing distinctive *in vivo* characteristics. The unique characteristics of these cells provide an opportunity to analyze the process of membranous ossification and endochondral ossification. These cells are useful candidate cell sources, in addition to dedifferentiated chondrocytes obtained from cartilage for transplantation in osteoarthritis and rheumatoid arthritis.

GENE EXPRESSION PROFILING AND MEDICAL SCIENCE

Recently, gene expression profiling has been successfully used to predict outcomes in some types of malignant diseases [55-61] and, additionally, to assess drug discovery screening [62]. In reproductive and regenerative medicine, it is important to identify biomarkers that will establish the isolation, selection and expansion of stem cells *in vitro* to allow their use for cell therapy. On the road map for translational medicine-- often referred to as bench to bedside research--, stem cell therapy is a prime destination. Stem cells have not taken on the identity of any specific cell type and are not yet committed to any dedicated function; they can divide extensively or indefinitely, and may be induced to give rise to one or more specialized cell types. Stem cells derived from bone marrow can replace heart muscle lost as a result of a heart attack, and can improve cardiac function. Injecting bone-marrow stem cells into an injured heart potentially represented new therapy, triggering the launch of numerous clinical studies to investigate the effect of directly injecting these cells into the damaged heart muscle of patients following a

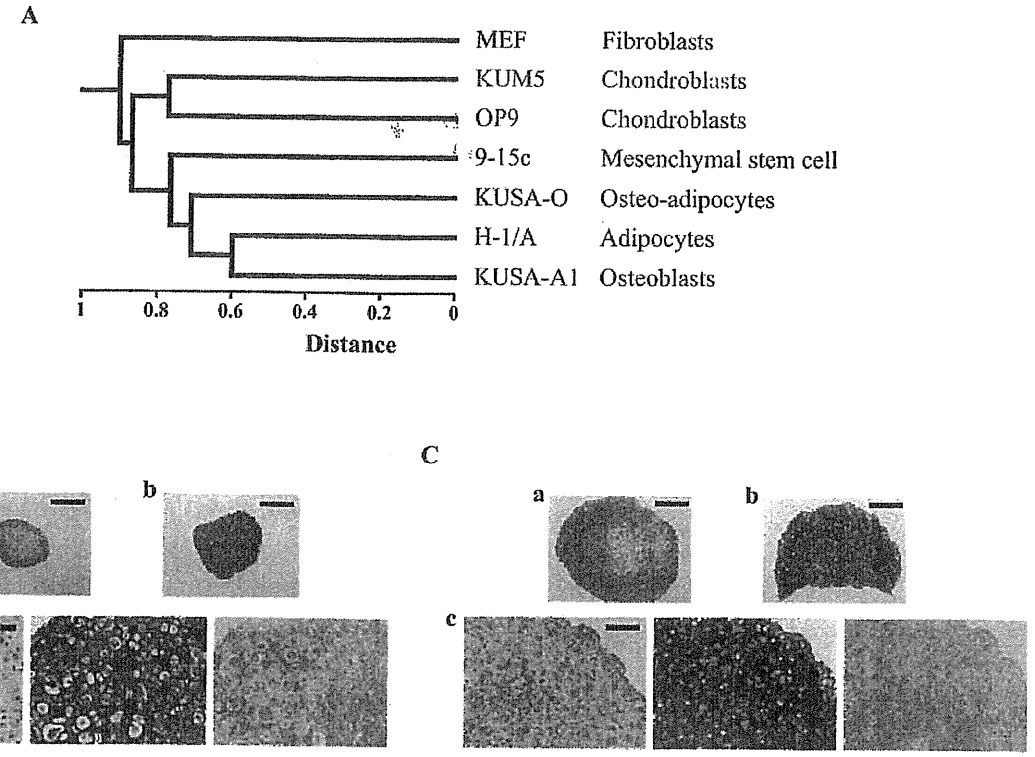

Fig. (3). Expression profiling and *in vitro* chondrogenesis of KUM5 and OP9 cells.

A. Dendrogram revealing clustering profile of six marrow stromal cells and mouse embryonic fibroblast (MEF) using 244 surface marker genes.

B. a, b: Toluidine blue stained section of KUM5 chondrogenic nodules in the pellet culture exposed TGF-β3 and BMP-2 for 1 (a) or 3 (b) weeks. Scale bars: 500 μm. c: Higher magnification of KUM5 chondrogenic pellet exposed to TGF-β3 and BMP-2 for 3 weeks. Left panel: hematoxylin and eosin stain; center panel: toluidine blue stain; right panel: alcian blue stain. Scale bars: 100 μm.

C. a, b: Toluidine blue stained section of OP9 chondrogenic nodules in the pellet culture exposed TGF-β3 and BMP-2 for 1 (a) or 3 (b) weeks. Scale bars: 500 μm. c: Higher magnification of OP9 chondrogenic pellet exposed to TGF-β3 and BMP-2 for 3 weeks. Left panel: hematoxylin and eosin stain; center panel: toluidine blue stain; right panel: alcian blue stain. Scale bars: 100 μm.

(Modified from Sugiki *et al.* J Cell Biochem, 2007, 100, 1240 [48]).

heart attack [63]. The scientific underpinnings of the ongoing human studies have been established. Now is the time to search for the presence of naturally occurring, authentic pluripotent cells and to identify and dissect the signals that guide their migration, self-renewal and differentiation. Furthermore, we need to commit the necessary time and resources to identify the best stem cells for cell therapy to translate.

CONCLUSION

Here, the expression pattern has been correlated with molecular structure descriptor; this consistency indicates that the expression profiling is valid. Consequently, understanding the global gene network that governs the pluripotency and self-renewal of stem cells is an important first step towards the experimental manipulation of cellular developmental potency. The cell potency is a fundamental concept in developmental biology and stem cell biology, providing a conceptual framework of sequential transition from totipotent fertilized eggs to pluripotent embryonic stem cells and stem cells to terminal differentiated cells. The global expression profiling can help to delineate the global architecture and dynamics of a gene regulatory network such as Oct4-regulated gene networks in mouse ES cells [64].

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FOOTNOTE

Fig. (1) is prepared from ref. [18] with permission from Elsevier.

Fig. (3) is prepared from ref. [48] with permission from Wiley-Liss, Inc.

REFERENCES

[1] Bryder, D.; Rossi, D.J.; Weissman, I.L. Hematopoietic stem cells: the paradigmatic tissue-specific stem cell. *Am. J. Pathol.*, **2006**, *169*, 338-346.

[2] Alison, M.; Sarraf, C. Hepatic stem cells. *J. Hepatol.*, **1998**, *29*, 676-682.

[3] Watt, F.M. Epidermal stem cells: markers, patterning and the control of stem cell fate. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.*, **1998**, *353*, 831-837.

[4] Potten, C.S. Stem cells in gastrointestinal epithelium: numbers, characteristics and death. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.*, **1998**, *353*, 821-830.

[5] Gage, F.H. Mammalian neural stem cells. *Science*, **2000**, *287*, 1433-1438.

[6] Jiang, Y.; Vaessen, B.; Lenvik, T.; Blackstad, M.; Reyes, M.; Verfaillie, C.M. Multipotent progenitor cells can be isolated from postnatal murine bone marrow, muscle, and brain. *Exp. Hematol.*, **2002**, *30*, 896-904.

[7] Jiang, Y.; Jahagirdar, B.N.; Reinhardt, R.L.; Schwartz, R.E.; Keene, C.D.; Ortiz-Gonzalez, X.R.; Reyes, M.; Lenvik, T.; Lund, T.; Blackstad, M.; Du, J.; Aldrich, S.; Lisberg, A.; Low, W.C.; Largaespada, D.A.; Verfaillie, C.M. Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature*, **2002**, *418*, 41-49.

[8] Fridenshtein, A. [Stromal bone marrow cells and the hematopoietic microenvironment]. *Arkh. Patol.*, **1982**, *44*, 3-11.

[9] Ko, M.S. Embryogenomics: developmental biology meets genomics. *Trends Biotechnol.*, **2001**, *19*, 511-518.

[10] Ivanova, N.B.; Dimos, J.T.; Schaniel, C.; Hackney, J.A.; Moore, K.A.; Lemischka, I.R. A stem cell molecular signature. *Science*, **2002**, *298*, 601-604.

[11] Ramalho-Santos, M.; Yoon, S.; Matsuzaki, Y.; Mulligan, R.C.; Melton, D.A. "Stemness": transcriptional profiling of embryonic and adult stem cells. *Science*, **2002**, *298*, 597-600.

[12] Schena, M.; Shalon, D.; Davis, R.W.; Brown, P.O. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science*, **1995**, *270*, 467-470.

[13] Tanaka, T.S.; Kunath, T.; Kimber, W.L.; Jaradat, S.A.; Stagg, C.A.; Usuda, M.; Yokota, T.; Niwa, H.; Rossant, J.; Ko, M.S. Gene expression profiling of embryo-derived stem cells reveals candidate genes associated with pluripotency and lineage specificity. *Genome Res.*, **2002**, *12*, 1921-1928.

[14] Brown, P.O.; Botstein, D. Exploring the new world of the genome with DNA microarrays. *Nat. Genet.*, **1999**, *21*, 33-37.

[15] DeRisi, J.L.; Iyer, V.R.; Brown, P.O. Exploring the metabolic and genetic control of gene expression on a genomic scale. *Science*, **1997**, *278*, 680-686.

[16] Iyer, V.R.; Eisen, M.B.; Ross, D.T.; Schuler, G.; Moore, T.; Lee, J.C.; Trent, J.M.; Staudt, L.M.; Hudson, J., Jr.; Boguski, M.S.; Lashkari, D.; Shalon, D.; Botstein, D.; Brown, P.O. The transcriptional program in the response of human fibroblasts to serum. *Science*, **1999**, *283*, 83-87.

[17] Carter, M.G.; Hamatani, T.; Sharov, A.A.; Carmack, C.E.; Qian, Y.; Aiba, K.; Ko, N.T.; Dudekula, D.B.; Brzoska, P.M.; Hwang, S.S.; Ko, M.S. In situ-synthesized novel microarray optimized for mouse stem cell and early developmental expression profiling. *Genome Res.*, **2003**, *13*, 1011-1021.

[18] Hamatani, T.; Carter, M.G.; Sharov, A.A.; Ko, M.S. Dynamics of global gene expression changes during mouse preimplantation development. *Dev Cell*, **2004**, *6*, 117-131.

[19] Ashburner, M.; Ball, C.A.; Blake, J.A.; Botstein, D.; Butler, H.; Cherry, J.M.; Davis, A.P.; Dolinski, K.; Dwight, S.S.; Eppig, J.T.; Harris, M.A.; Hill, D.P.; Issel-Tarver, L.; Kasarskis, A.; Lewis, S.; Matese, J.C.; Richardson, J.E.; Ringwald, M.; Rubin, G.M.; Sherlock, G. Gene ontology: tool for the unification of biology. The gene ontology consortium. *Nat. Genet.*, **2000**, *25*, 25-29.

[20] Dahlquist, K.D.; Salomonis, N.; Vranizan, K.; Lawlor, S.C.; Conklin, B.R. GenMAPP, a new tool for viewing and analyzing microarray data on biological pathways. *Nat. Genet.*, **2002**, *31*, 19-20.

[21] Doniger, S.W.; Salomonis, N.; Dahlquist, K.D.; Vranizan, K.; Lawlor, S.C.; Conklin, B.R. MAPPFinder: using Gene Ontology and GenMAPP to create a global gene-expression profile from microarray data. *Genome Biol.*, **2003**, *4*, R7.

[22] Hamatani, T.; Falco, G.; Carter, M.G.; Akutsu, H.; Stagg, C.A.; Sharov, A.A.; Dudekula, D.B.; VanBuren, V.; Ko, M.S. Age-associated alteration of gene expression patterns in mouse oocytes. *Hum. Mol. Genet.*, **2004**, *13*, 2263-2278.

[23] Pan, H.; Ma, P.; Zhu, W.; Schultz, R.M. Age-associated increase in aneuploidy and changes in gene expression in mouse eggs. *Dev. Biol.*, **2008**, *316*, 397-407.

[24] Strutz, F.; Okada, H.; Lo, C.W.; Danoff, T.; Carone, R.L.; Tomaszewski, J.E.; Neilson, E.G. Identification and characterization of a fibroblast marker: FSP1. *J. Cell Biol.*, **1995**, *130*, 393-405.

[25] Iwano, M.; Plieth, D.; Danoff, T.M.; Xue, C.; Okada, H.; Neilson, E.G. Evidence that fibroblasts derive from epithelium during tissue fibrosis. *J. Clin. Invest.*, **2002**, *110*, 341-350.

[26] Janda, E.; Lehmann, K.; Killisch, I.; Jechlinger, M.; Herzig, M.; Downward, J.; Beug, H.; Grunert, S. Ras and TGF[beta] cooperatively regulate epithelial cell plasticity and metastasis: dissection of Ras signaling pathways. *J. Cell Biol.*, **2002**, *156*, 299-313.

[27] Kiemer, A.K.; Takeuchi, K.; Quinlan, M.P. Identification of genes involved in epithelial-mesenchymal transition and tumor progression. *Oncogene*, **2001**, *20*, 6679-6688.

[28] Vincent-Salomon, A.; Thiery, J.P. Host microenvironment in breast cancer development: epithelial-mesenchymal transition in breast cancer development. *Breast Cancer Res*, **2003**, *5*, 101-106.

[29] Xue, C.; Plieth, D.; Venkov, C.; Xu, C.; Neilson, E.G. The gatekeeper effect of epithelial-mesenchymal transition regulates the frequency of breast cancer metastasis. *Cancer Res.*, **2003**, *63*, 3386-3394.

[30] Ferrari, G.; Cusella-De Angelis, G.; Coletta, M.; Paolucci, E.; Stornaiuolo, A.; Cossu, G.; Mavilio, F. Muscle regeneration by bone marrow-derived myogenic progenitors. *Science*, **1998**, *279*, 1528-1530.

[31] Krause, D.S.; Theise, N.D.; Collector, M.I.; Henegariu, O.; Hwang, S.; Gardner, R.; Neutzel, S.; Sharkis, S.J. Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. *Cell*, **2001**, *105*, 369-377.

[32] Petersen, B.E.; Bowen, W.C.; Patrene, K.D.; Mars, W.M.; Sullivan, A.K.; Murase, N.; Boggs, S.S.; Greenberger, J.S.; Goff, J.P. Bone marrow as a potential source of hepatic oval cells. *Science*, **1999**, *284*, 1168-1170.

[33] Sanchez-Ramos, J.; Song, S.; Cardozo-Pelaez, F.; Hazzi, C.; Stedeford, T.; Willing, A.; Freeman, T.B.; Saporta, S.; Janssen, W.; Patel, N.; Cooper, D.R.; Sanberg, P.R. Adult bone marrow stromal cells differentiate into neural cells *in vitro*. *Exp. Neurol.*, **2000**, *164*, 247-256.

[34] Woodbury, D.; Schwarz, E.J.; Prockop, D.J.; Black, I.B. Adult rat and human bone marrow stromal cells differentiate into neurons. *J. Neurosci. Res.*, **2000**, *61*, 364-370.

[35] Kohyama, J.; Abe, H.; Shimazaki, T.; Koizumi, A.; Nakashima, K.; Gojo, S.; Taga, T.; Okano, H.; Hata, J.; Umezawa, A. Brain from bone: efficient "meta-differentiation" of marrow stroma-derived mature osteoblasts to neurons with Noggin or a demethylating agent. *Differentiation*, **2001**, *68*, 235-244.

[36] Kim, B.J.; Seo, J.H.; Bubien, J.K.; Oh, Y.S. Differentiation of adult bone marrow stem cells into neuroprogenitor cells *in vitro*. *Neuroreport*, **2002**, *13*, 1185-1188.

[37] Solursh, M. Differentiation of cartilage and bone. *Curr. Opin. Cell Biol.*, **1989**, *1*, 989-994.

[38] Hauselmann, H.J.; Fernandes, R.J.; Mok, S.S.; Schmid, T.M.; Block, J.A.; Aydelotte, M.B.; Kuettner, K.E.; Thonar, E.J. Phenotypic stability of bovine articular chondrocytes after long-term culture in alginate beads. *J. Cell Sci.*, **1994**, *107 (Pt 1)*, 17-27.

[39] Reginato, A.M.; Iozzo, R.V.; Jimenez, S.A. Formation of nodular structures resembling mature articular cartilage in long-term primary cultures of human fetal epiphyseal chondrocytes on a hydrogel substrate. *Arthritis Rheum.*, **1994**, *37*, 1338-1349.

[40] Archer, C.W.; McDowell, J.; Bayliss, M.T.; Stephens, M.D.; Bentley, G. Phenotypic modulation in sub-populations of human articular chondrocytes *in vitro*. *J. Cell Sci.*, **1990**, *97(Pt 2)*, 361-371.

[41] Benya, P.D.; Padilla, S.R.; Nimni, M.E. Independent regulation of collagen types by chondrocytes during the loss of differentiated function in culture. *Cell,* **1978**, *15*, 1313-1321.

[42] Benya, P.D.; Shaffer, J.D. Dedifferentiated chondrocytes reexpress the differentiated collagen phenotype when cultured in agarose gels. *Cell,* **1982**, *30*, 215-224.

[43] Lefebvre, V.; Peeters-Joris, C.; Vaes, G. Production of collagens, collagenase and collagenase inhibitor during the dedifferentiation of articular chondrocytes by serial subcultures. *Biochim. Biophys. Acta,* **1990**, *1051*, 266-275.

[44] Bonaventure, J.; Kadhom, N.; Cohen-Solal, L.; Ng, K.H.; Bourguignon, J.; Lasselin, C.; Freisinger, P. Reexpression of cartilage-specific genes by dedifferentiated human articular chondrocytes cultured in alginate beads. *Exp. Cell Res.,* **1994**, *212*, 97-104.

[45] Yoon, Y.M.; Kim, S.J.; Oh, C.D.; Ju, J.W.; Song, W.K.; Yoo, Y.J.; Huh, T.L.; Chun, J.S. Maintenance of differentiated phenotype of articular chondrocytes by protein kinase C and extracellular signal-regulated protein kinase. *J. Biol. Chem.,* **2002**, *277*, 8412-8420.

[46] Matsumoto, S.; Shibuya, I.; Kusakari, S.; Segawa, K.; Uyama, T.; Shimada, A.; Umezawa, A. Membranous osteogenesis system modeled with KUSA-A1 mature osteoblasts. *Biochim. Biophys. Acta,* **2005**, *1725*, 57-63.

[47] Umezawa, A.; Maruyama, T.; Segawa, K.; Shadduck, R.K.; Waheed, A.; Hata, J. Multipotent marrow stromal cell line is able to induce hematopoiesis *in vivo*. *J. Cell. Physiol.,* **1992**, *151*, 197-205.

[48] Sugiki, T.; Uyama, T.; Toyoda, M.; Morioka, H.; Kume, S.; Miyado, K.; Matsumoto, K.; Saito, H.; Tsumaki, N.; Takahashi, Y.; Toyama, Y.; Umezawa, A. Hyaline cartilage formation and enchondral ossification modeled with KUM5 and OP9 chondroblasts. *J. Cell. Biochem.,* **2007**, *100*, 1240-1254.

[49] Nakano, T.; Kodama, H.; Honjo, T. *In vitro* development of primitive and definitive erythrocytes from different precursors. *Science,* **1996**, *272*, 722-724.

[50] Kodama, H.; Nose, M.; Niida, S.; Nishikawa, S. Involvement of the c-kit receptor in the adhesion of hematopoietic stem cells to stromal cells. *Exp. Hematol.,* **1994**, *22*, 979-984.

[51] Nakano, T.; Kodama, H.; Honjo, T. Generation of lymphohematopoietic cells from embryonic stem cells in culture. *Science,* **1994**, *265*, 1098-1101.

[52] Nakano, T. Lymphohematopoietic development from embryonic stem cells *in vitro*. *Semin. Immunol.,* **1995**, *7*, 197-203.

[53] Calvi, L.M.; Adams, G.B.; Weibrecht, K.W.; Weber, J.M.; Olson, D.P.; Knight, M.C.; Martin, R.P.; Schipani, E.; Divieti, P.; Bringhurst, F.R.; Milner, L.A.; Kronenberg, H.M.; Scadden, D.T. Osteoblastic cells regulate the haematopoietic stem cell niche. *Nature,* **2003**, *425*, 841-846.

[54] Zhang, J.; Niu, C.; Ye, L.; Huang, H.; He, X.; Tong, W.G.; Ross, J.; Haug, J.; Johnson, T.; Feng, J.Q.; Harris, S.; Wiedemann, L.M.; Mishina, Y.; Li, L. Identification of the haematopoietic stem cell niche and control of the niche size. *Nature,* **2003**, *425*, 836-841.

[55] Alizadeh, A.A.; Eisen, M.B.; Davis, R.E.; Ma, C.; Lossos, I.S.; Rosenwald, A.; Boldrick, J.C.; Sabet, H.; Tran, T.; Yu, X.; Powell, J.I.; Yang, L.; Marti, G.E.; Moore, T.; Hudson, J., Jr.; Lu, L.; Lewis, D.B.; Tibshirani, R.; Sherlock, G.; Chan, W.C.; Greiner, T.C.; Weisenburger, D.D.; Armitage, J.O.; Warnke, R.; Levy, R.; Wilson, W.; Grever, M.R.; Byrd, J.C.; Botstein, D.; Brown, P.O.; Staudt, L.M. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature,* **2000**, *403*, 503-511.

[56] Beer, D.G.; Kardia, S.L.; Huang, C.C.; Giordano, T.J.; Levin, A.M.; Misek, D.E.; Lin, L.; Chen, G.; Gharib, T.G.; Thomas, D.G.; Lizyness, M.L.; Kuick, R.; Hayasaka, S.; Taylor, J.M.; Iannettoni, M.D.; Orringer, M.B.; Hanash, S. Gene-expression profiles predict survival of patients with lung adenocarcinoma. *Nat. Med.,* **2002**, *8*, 816-824.

[57] Pomeroy, S.L.; Tamayo, P.; Gaasenbeek, M.; Sturla, L.M.; Angelo, M.; McLaughlin, M.E.; Kim, J.Y.; Goumnerova, L.C.; Black, P.M.; Lau, C.; Allen, J.C.; Zagzag, D.; Olson, J.M.; Curran, T.; Wetmore, C.; Biegel, J.A.; Poggio, T.; Mukherjee, S.; Rifkin, R.; Califano, A.; Stolovitzky, G.; Louis, D.N.; Mesirov, J.P.; Lander, E.S.; Golub, T.R. Prediction of central nervous system embryonal tumour outcome based on gene expression. *Nature,* **2002**, *415*, 436-442.

[58] Rosenwald, A.; Wright, G.; Chan, W.C.; Connors, J.M.; Campo, E.; Fisher, R.I.; Gascoyne, R.D.; Muller-Hermelink, H.K.; Smeland, E.B.; Giltnane, J.M.; Hurt, E.M.; Zhao, H.; Averett, L.; Yang, L.; Wilson, W.H.; Jaffe, E.S.; Simon, R.; Klausner, R.D.; Powell, J.; Duffey, P.L.; Longo, D.L.; Greiner, T.C.; Weisenburger, D.D.; Sanger, W.G.; Dave, B.J.; Lynch, J.C.; Vose, J.; Armitage, J.O.; Montserrat, E.; Lopez-Guillermo, A.; Grogan, T.M.; Miller, T.P.; LeBlanc, M.; Ott, G.; Kvaloy, S.; Delabie, J.; Holte, H.; Krajci, P.; Stokke, T.; Staudt, L.M. The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. *N. Engl. J. Med.,* **2002**, *346*, 1937-1947.

[59] Shipp, M.A.; Ross, K.N.; Tamayo, P.; Weng, A.P.; Kutok, J.L.; Aguiar, R.C.; Gaasenbeek, M.; Angelo, M.; Reich, M.; Pinkus, G.S.; Ray, T.S.; Koval, M.A.; Last, K.W.; Norton, A.; Lister, T.A.; Mesirov, J.; Neuberg, D.S.; Lander, E.S.; Aster, J.C.; Golub, T.R. Diffuse large B-cell lymphoma outcome prediction by gene-expression profiling and supervised machine learning. *Nat. Med.,* **2002**, *8*, 68-74.

[60] van 't Veer, L.J.; Dai, H.; van de Vijver, M.J.; He, Y.D.; Hart, A.A.; Mao, M.; Peterse, H.L.; van der Kooy, K.; Marton, M.J.; Witteveen, A.T.; Schreiber, G.J.; Kerkhoven, R.M.; Roberts, C.; Linsley, P.S.; Bernards, R.; Friend, S.H. Gene expression profiling predicts clinical outcome of breast cancer. *Nature,* **2002**, *415*, 530-536.

[61] Yeoh, E.J.; Ross, M.E.; Shurtleff, S.A.; Williams, W.K.; Patel, D.; Mahfouz, R.; Behm, F.G.; Raimondi, S.C.; Relling, M.V.; Patel, A.; Cheng, C.; Campana, D.; Wilkins, D.; Zhou, X.; Li, J.; Liu, H.; Pui, C.H.; Evans, W.E.; Naeve, C.; Wong, L.; Downing, J.R. Classification, subtype discovery, and prediction of outcome in pediatric acute lymphoblastic leukemia by gene expression profiling. *Cancer Cell,* **2002**, *1*, 133-143.

[62] Scherf, U.; Ross, D.T.; Waltham, M.; Smith, L.H.; Lee, J.K.; Tanabe, L.; Kohn, K.W.; Reinhold, W.C.; Myers, T.G.; Andrews, D.T.; Scudiero, D.A.; Eisen, M.B.; Sausville, E.A.; Pommier, Y.; Botstein, D.; Brown, P.O.; Weinstein, J.N. A gene expression database for the molecular pharmacology of cancer. *Nat. Genet.,* **2000**, *24*, 236-244.

[63] Chien, K.R. Stem cells: lost in translation. *Nature,* **2004**, *428*, 607-608.

[64] Matoba, R.; Niwa, H.; Masui, S.; Ohtsuka, S.; Carter, M.G.; Sharov, A.A.; Ko, M.S. Dissecting Oct3/4-regulated gene networks in embryonic stem cells by expression profiling. *PLoS One,* **2006**, *1*, e26.

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REPRODUCTIVE BIOLOGY AND ENDOCRINOLOGY

REVIEW Open Access

Acute stress may induce ovulation in women

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Abstract

Background: This study aims to gather information either supporting or rejecting the hypothesis that acute stress may induce ovulation in women. The formulation of this hypothesis is based on 2 facts: 1) estrogen-primed postmenopausal or ovariectomized women display an adrenal-progesterone-induced ovulatory-like luteinizing hormone (LH) surge in response to exogenous adrenocorticotropic hormone (ACTH) administration; and 2) women display multiple follicular waves during an interovulatory interval, and likely during pregnancy and lactation. Thus, acute stress may induce ovulation in women displaying appropriate serum levels of estradiol and one or more follicles large enough to respond to a non-midcycle LH surge.

Methods: A literature search using the PubMed database was performed to identify articles up to January 2010 focusing mainly on women as well as on rats and rhesus monkeys as animal models of interaction between the hypothalamic-pituitary-adrenal (HPA) and hypothalamic-pituitary-gonadal (HPG) axes.

Results: Whereas the HPA axis exhibits positive responses in practically all phases of the ovarian cycle, acute-stress-induced release of LH is found under relatively high plasma levels of estradiol. However, there are studies suggesting that several types of acute stress may exert different effects on pituitary LH release and the steroid environment may modulate in a different way (inhibiting or stimulating) the pattern of response of the HPG axis elicited by acute stressors.

Conclusion: Women may be induced to ovulate at any point of the menstrual cycle or even during periods of amenorrhea associated with pregnancy and lactation if exposed to an appropriate acute stressor under a right estradiol environment.

Background

It is known that the percentage of pregnancies resulting from single episodes of forced penile-vaginal intercourse (rape) is significantly higher (8.0% in a sample of 405 women from a national random-digit dialing sample of households in USA) than the percentage of pregnancies resulting from single episodes of consensual, unprotected intercourse (3.1% in a sample of 221 women with no fertility problems planning to become pregnant in USA) [1]. It is worth mentioning that data from the study by Gottschall and Gottschall [1] were adjusted for the use of oral contraception and intra-uterine devices (IUDs). Furthermore, Gottschall and Gottschall [1] elegantly ruled out the possibility that in their study higher rape-pregnancy rates may result from (1) women being more likely to report to medical or law-enforcement authorities rapes resulting in conception; (2) women sometimes attributing paternity to rapists when they were fertilized by a consensual partner; and (3) a high number of rape victims coming from the most fecund age cohorts of the population, i.e. rapists disproportionately would target young women in their most fecund years. These points were the main grounds used in previous studies to reject the fact that per-incident rape-pregnancy risk had been reiteratively reported to be higher than per-incident consensual pregnancy risk.

It is also known that, in fertile women planning to become pregnant, ovulation and conception may occur on any day of the menstrual cycle, although the maximum probability is reached at the middle of the cycle [2]. Furthermore, ovulations and conceptions may arise during periods of amenorrhea associated with oral contraceptive use, drug addiction, pregnancy (superfetation; for review, see Pape et al. [3]) and lactation. In addition, fertilization of ≥ 2 oocytes from the same menstrual cycle by

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sperm from separate acts of sexual intercourse has been also reported (superfecundation [4,5]).

All this epidemiological evidence, together with the fact that copulation can trigger or hasten (facilitate) ovulation in otherwise spontaneous ovulating species such as the rat (for reviews, see Gibson et al. [6], Milligan [7], Bakker and Baum [8] and Nagy et al. [9]), led Zarrow et al. [10] and Jöchle [11,12] to propose that women may be facultative coitus-induced ovulators.

This attractive and stimulating hypothesis is supported by the fact that 68% of women display 2 follicular waves and the remaining 32% exhibit 3 waves of ovarian follicular development during an interovulatory interval [13]. It is likely that women exhibit waves of follicular development during pregnancy and lactation as it occurs in cattle, sheep, goats and mares [14] (for review, see Evans [15]).

The presence of multiple follicular waves in women may provide an extra source of oocytes to be ovulated if an appropriate non-midcycle luteinizing hormone (LH) surge took place. We should note that follicular waves can be either ovulatory (the final wave of follicular development) or anovulatory (all the preceding waves) [13,16]. Twenty one percent of the anovulatory follicular waves are major, i.e. those in which one follicle is selected to be dominant over other follicles of the wave, and the remaining 79% minor, i.e. those in which no selection of a dominant follicle is evidenced. Although, anovulatory follicles do not develop as large as ovulatory follicles, they grow to an ostensible preovulatory diameter before undergoing atresia (maximum diameter of the dominant follicle of an anovulatory major wave: 12.9 ± 0.8 mm, range 10-17 mm; maximum diameter of the largest follicle of an anovulatory minor wave: 8.1 ± 0.1 mm, range 7-9 mm) [17]. The maximum diameters reached by dominant follicles from anovulatory major waves (range 10-17 mm) are compatible with potential ovulations if follicles are correctly stimulated. In fact, spontaneous ovulations in normal menstrual cycles have been reported to occur in follicles ≥ 15 mm in diameter [16].

Despite the fact that women display multiple waves of follicular development during an interovulatory interval, and likely during pregnancy and lactation, the hypothesis that women may be coitus-induced ovulators is directly refuted by studies showing that neither coitus nor orgasm induces a surge in LH secretion (and thus ovulation) in women [18-20] and rhesus monkeys [21]. Results from these classical studies have been recently endorsed by Baerwald et al. [13,16]. These authors tracked daily the follicle diameter and follicle number present in the ovaries of 50 healthy women of reproductive age (28.0 ± 6.9, range 19-43 years) displaying clinically normal menstrual cycles and not taking medications known to interfere with reproductive function. The ovarian ultrasonographic examinations only evidenced ovulations in follicles from the last wave of the interovulatory cycle, which emerges at the early follicular phase. The preceding waves, emerging at the luteal phase, were anovulatory in all 50 women entered into the study. This fact contradicts the assumption that coitus may induce ovulation in women. We should bear in mind that, in addition to a periovulatory peak, human beings display no changes at all or even rises in male- and female-initiated sexual activity, woman's sexual desire, autosexual activity and sexual arousability, and interpersonal sexual activities, including sexual intercourse, during the mid-follicular and late-luteal phases (for review, see Tarín and Gómez-Piquer [22]). Thus, it is expected that a non-negligible number of the 50 women analyzed by Baerwald et al. [13,16] was presumably engaged in sexual intercourse during the period of ultrasonographic evaluations (one interovulatory cycle).

If we consider the laboratory rat as a paradigm of facultative coitus-induced ovulation, it can be noted that there are notable differences between women and rats in the neuroendocrine mechanisms controlling the ovarian cycle. For instance, in contrast to women, both spontaneous and induced ovulatory mechanisms are integrated in the rat. Female rats have no functional corpora lutea and must receive vulval, vaginal and/or cervical intromissive stimulation in order for the ovaries to develop fully functional corpora lutea. Mating stimulates the release of prolactin from the anterior pituitary, which is required for activation of the corpora lutea and progesterone biosynthesis (for review, see Bakker and Baum [8]). Furthermore, although technical difficulties have precluded determining the ovarian dynamics in rats by transcutaneous ultrasound bio-microscopy [23], it is expected that rats exhibit a single wave of follicular development such as evidenced in the mouse [23] and the coitus-induced ovulating species analyzed as of today, including cats, llamas and camels (for review, see Evans [15]).

Interaction of the hypothalamic-pituitary-adrenal (HPA) and hypothalamic-pituitary-gonadal (HPG) axes

While the woman's reproductive physiology does not fit the rat paradigm of facultative coitus-induced ovulation, the HPA and HPG axes interact with each other in a similar way in both species. Indeed, in rats and women, there is a positive coupling between the HPA axis and the HPG axis at the time of the preovulatory LH surge. In women, initiation of the LH surge takes place either at 04.00 a.m. in 20% of the cases or at 08.00 a.m. in the remaining 80% of the cases. The onset of the LH surge is strongly coupled with the time at which the peak (acrophase) of the cortisol circadian rhythm occurs, i.e. maximal cortisol plasma concentrations take place at 04.00 a.m. when the LH preovulatory surge initiates at 04.00 a.m. and at 08.00

a.m. when the LH preovulatory surge starts at 08.00 a.m. [24]. Likewise, in the laboratory rat, both the preovulatory LH surge and the maximal plasma values of the corticosterone circadian rhythm are observed during the transition from the light to the dark phase (2 h preceding and 2 h following) on the day of proestrus [25,26].

Moreover, it is well-known that prolonged or chronic stress in rats and women may block, inhibit or delay the preovulatory LH surge and therefore disrupt the estrous or menstrual cycle (for reviews, see Rivier and Rivest [27] and Kalantaridou et al. [28]). On the contrary, women and rats exposed to acute stress may respond with an adrenal-progesterone-induced LH surge (for review, see Mahesh and Brann [29]).

There is considerable evidence in rats and women showing an elevation in the levels of serum progesterone mainly from the adrenal glands prior to the onset of the LH surge. This increase in progesterone levels serves to initiate, synchronize, potentiate and limit the preovulatory LH surge to a single day (for review, see Mahesh and Brann [29]). In order for progesterone to exert its facilitatory role on gonadotropin secretion, the presence of a background of high estradiol is essential [30]. This is due to the fact that estradiol induces the expression of anterior pituitary, hypothalamic and extrahypothalamic progesterone receptors, which function as transcriptional regulators that prompt alterations in gene expression needed for facilitation of neurosecretion of gonadotropin-releasing hormone (GnRH) surges and release of periovulatory gonadotropin surges (for reviews, see Mahesh and Brann [29] and Levine et al. [31]).

It is not surprising, therefore, that progesterone and natural mineralocorticoids, such as deoxycorticosterone, and synthetic glucocorticoids, such as triamcinolone acetonide, which possess "progestin-like" activity, stimulate the release of LH and follicle-stimulating hormone (FSH) when administered acutely to pregnant mare serum gonadotropin (PMSG)-primed immature [32] or estrogen-primed ovariectomized immature rats [33]. In estrogen-treated menopausal women, it has been demonstrated that progesterone administration is able to induce a pre-ovulatory-type surge of LH and FSH (for review, see Mahesh and Brann [29]. These findings have led to propose that the improvement in menstrual rhythm and ovulatory activity following glucocorticoid therapy in women suffering from polycystic ovarian syndrome or other syndromes of androgen excess may be due to its direct effects on the release of gonadotropins, in addition to the ability of glucocorticoids to suppress adrenal overproduction of androgens [32,33].

Moreover, it has been reported that a single injection of adrenocorticotropic hormone (ACTH) to estrogen-primed intact and ovariectomized immature rats causes a significant elevation in serum LH and FSH levels. However, this ACTH treatment fails to induce a surge of gonadotropins in non-estrogen-primed intact immature rats. It is worth mentioning that of the 2 major adrenal steroids secreted as result of ACTH administration, i.e. progesterone and corticosterone, only progesterone is able to stimulate LH release in estrogen-primed ovariectomized immature rats on the day of its administration [34]. Likewise, a 3-h intravenous infusion of ACTH to postmenopausal or ovariectomized women with estrogen replacement raises the plasma levels of corticosterone and progesterone 3-4 h after injection, accompanied by a significant stimulation of LH release 2-3 h after the initial raise in progesterone [35].

Aim of the study

The aim of this bioessay is to gather information either supporting or rejecting the hypothesis that acute stress may induce ovulation in women. The formulation of this hypothesis is based on 2 facts: 1) estrogen-primed postmenopausal or ovariectomized women display an adrenal-progesterone-induced ovulatory-like LH surge in response to exogenous ACTH administration; and 2) women display multiple follicular waves during an interovulatory interval, and likely during pregnancy and lactation. Thus, acute stress may induce ovulation in women displaying appropriate serum levels of estradiol and one or more follicles large enough to respond to a non-midcycle LH surge.

Methods

As the HPA and HPG axes interact with each other in a similar way in rats and women, a literature search using the PubMed database [36] was performed to identify all the articles up to January 2010 dealing with the response of the HPA and HPG axes to acute stressors in rats and women. Studies on rhesus monkeys (*Macaca mulatta*) and previous reviews were also consulted. The following key words were used: "acute stress", "gonadotropin secretion", "luteinizing hormone surge", "hypothalamic-pituitary-adrenal axis", "hypothalamic-pituitary-gonadal axis", "premature luteinization" and "adrenal progesterone secretion".

Changes in serum levels of estradiol during the ovarian cycle in rats and women

As the serum concentration of estradiol in rats and women fluctuates during the estrous or menstrual cycle, it is expected that the LH-release response of the anterior pituitary to an acute-stress-induced surge of adrenal progesterone will vary during the estrous or menstrual cycle. Thus, in order to analyze the studies reviewed in this bioessay, it is important to recall the pattern of fluctuations in serum levels of estradiol during the ovarian cycle in both rats and women (female macaque monkeys have a