—Brief Note—

Spontaneous Parthenogenesis in Human Oocytes

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Abstract: *A total of 1,923 follicular human ova were collected for use in intracytoplasmic sperm injection (ICSI). Timed ovulation was induced by means of a standard ovarian stimulation protocol involving set dosages of follicle stimulating hormone (FSH), human menopausal gonadotrophin (HMG) and human chorionic gonadotrophin (hCG). Prior to performing ICSI, all of the ova were subjected to thorough examination which led to the discovery of only two parthenogenetic oocytes, also referred to as parthenogens, out of the total 1,923. These oocytes were retrieved from different patients. Although genetic analysis revealed that one of the parthenogens had a haploid number of chromosomes, it was impossible to analyze the second because it had not yet reached the metaphase stage. The 0.1 percent incidence of spontaneous parthenogenesis in this study clearly indicates that this phenomenon is extremely rare.*
Key words: *Human, Parthenogenesis, Chromosome, ICSI, Haploid.*

Although experimentally induced parthenogenesis has been well documented in the past, very little is understood about spontaneous parthenogenesis in mammals. The presence of parthenogenetic oocytes, also known as parthenogens, has been linked to the occurrence of ovarian teratoma [1]. The exact relationship between the two conditions however, as well as whether or not one plays a role in causing the other, is still unknown. In 1963, Yang and Hayashi [2] reported a single case of spontaneous parthenogenesis occurring in the ovarian follicle of a human subject. They concluded that naturally occurring parthenogenesis is extremely rare. The purpose of this study is not only to verify past findings but to document the chromosomal distribution in parthenogenetic oocytes as well.

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Oocyte collection and Cumulus removal: 1,923 oocytes were collected for 188 ICSI cycles between June, 1994 and April, 1995. Our standard ovarian stimulation protocol involved three days of follicle stimulating hormone (FSH) injections followed by three days of combined FSH/human menopausal gonadotrophin (HMG) injections and finally three days of HMG injections. Oocytes were retrieved via vaginal ultrasound-guided puncture 34–36 hr after the administration of human chorionic gonadotrophin (hCG). The corona radiata cells surrounding the oocyte were then removed with an injection needle. The cumulus cells were removed by incubation in a mixture of modified human tubal fluid (m-HTF) and 60 IU hyaluronidase/ml for 30 sec. Any remaining cumulus cells were removed by aspiration with a mouth pipet with an opening of 150 μm. Within two hours after cumulus removal, each oocyte was transferred to a Petri dish and immersed in 7 separate μl droplets of m-HTF which were later covered in mineral oil. This medium was maintained at a constant temperature of 37°C. Prior to ICSI, all oocytes were examined under an inverted microscope at a magnification of 300–450 times.

Chromosome analysis: Any oocyte that was found to be undergoing cleavage was then prepared for chromosome analysis by short term hypotonic treatment [3]. They were rinsed briefly with 1.0% sodium citrate and transferred to a well containing 0.4 ml of the same 1.0% sodium citrate solution. The total rinsing time in this treatment was approximately two to three minutes at room temperature. Under observation with a binocular microscope, a mild 0.03 ml acetic-alcohol fixative (1 part acetic acid to 3 parts methanol) was added to the hypotonic solution in which the oocyte was immersed. After placing the oocyte on a clean slide, five to seven drops (one drop equals 0.015 ml to 0.02 ml) of the

same acetic-alcohol fixative used in the previous step were applied to the oocyte. Following the second application of fixative, the oocyte was gently blown on and then dried on a hot plate at a temperature of 37°C to 38°C for two to three minutes. When the slide was completely dry, it was stained with a 2% Giemsa solution (in phosphate buffer, pH 6.8) for a period of ten minutes.

Out of the 1,923 oocytes studied, only two were found to be two-cell stage parthenogens. Each of these ova were retrieved from different patients. This figure (2/1,923) reflects a 0.1 percent incidence of spontaneous parthenogenesis (Table 1). One of the parthenogens was retrieved from a 29 year old woman (Case I, see Fig. 1), with a four year history of primary sterility. Case I had a regular 30 day menstrual cycle beginning with a six to seven day period of bleeding. The husband was diagnosed as oligozoospermic. The parthenogen was found among the six oocytes retrieved from this patient. The Case I parthenogen was globular with a diameter of 135 μm. The equatorial and longitudinal diameters of both blastomeres were 84 by 64 μm and 86 by 65 μm, respectively. One spherical polar body was found in the perivitellin space with a diameter of approximately 16 μm. The thickness of zona pellucida was observed to be 16 μm.

The other parthenogen was retrieved from a 35 year old woman (Case II, see Fig. 2) with a ten year history of primary sterility. Case II had a regular 30 day menstrual cycle beginning with a bleeding period of five days. Her husband was also diagnosed as oligozoospermic. This parthenogen was found among the seven oocytes extracted from patient II. The Case II parthenogen was globular with a diameter of 138 μm. The equatorial and longitudinal diameters of the blastomeres were found to be identical, both measuring 79 by 69 μm. One spherical polar body was found in the perivitellin space with a diameter of approximately 16 μm. The thickness of the zona pellucida was observed to be 16 μm.

Chromosome analysis of the Case I parthenogen determined it to be haploid (Fig. 3), with each blastomere having 23 chromosomes, but the chromosomes of the Case II parthenogen could not be analyzed as it did not enter metaphase.

While there are many studies dealing with the phenomenon of animal parthenogenesis, research on parthenogenesis occurring naturally in human ova has been scarce. The earliest observations of human parthenogenesis were made when tumorous ovaries were removed and examined histologically, but in 1971 Hayashi [4] observed parthenogenesis occurring in the human ovarian follicle prior to ovulation. In their report, the incidence of parthenogenesis was presented as

Table 1. Frequency of parthenogenesis in human oocytes

	Number (%) of		
Cycles	Oocytes retrieved	Metaphase II oocytes	Parthenogens
188	1,923	1,706	2/1,706 (0.1)

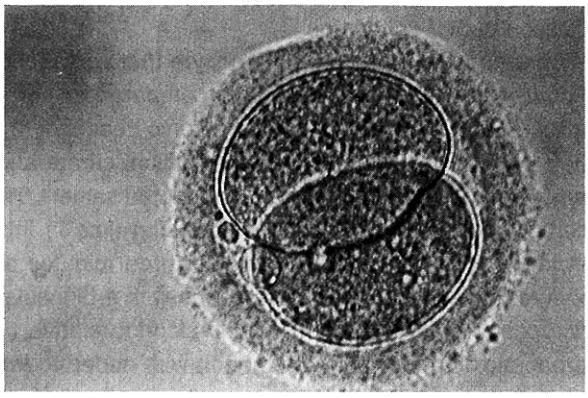

Fig. 1. Parthenogen from case I.

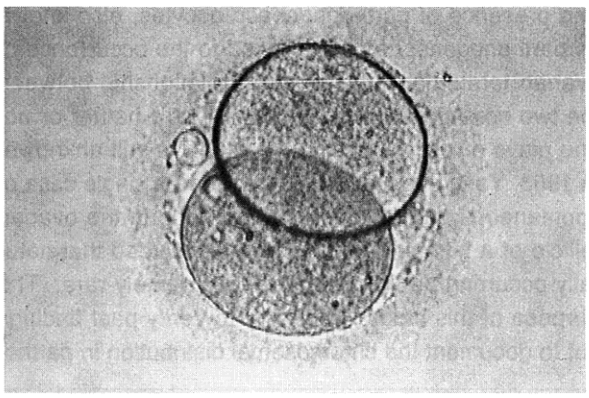

Fig. 2. Parthenogen from case II.

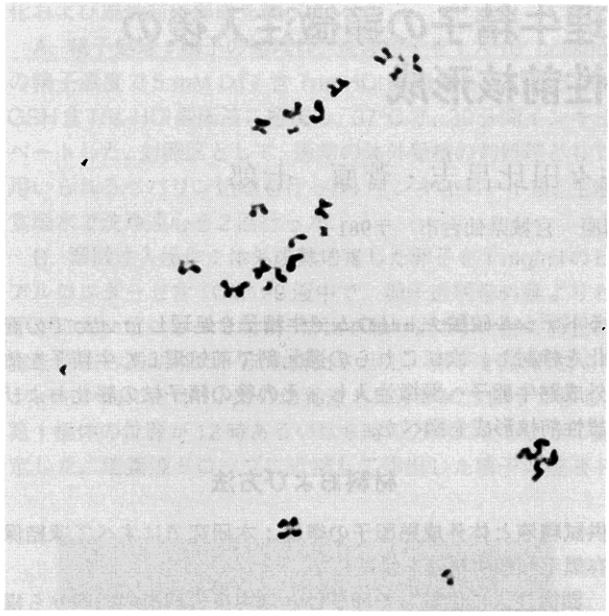

Fig. 3. A metaphase spread from an uninseminated human oocyte, obtained by the technique described in the materials and methods.

1.7%. The 0.1% rate obtained in the current study suggests an even lower actual frequency. The results of chromosome content analysis of the two parthenogens that were found provide concrete evidence demonstrating the natural occurrence of spontaneous parthenogenesis in humans. Whereas normally dividing oocytes exhibit two polar bodies in the perivitellin space following fertilization, each parthenogen in this study had only one observable polar body. It is quite possible that one of the oocyte's pronuclei, which would normally become a first polar body, remains within the cytoplasm and functions as a substitute sperm-pronucleus thereby facilitating parthenogenetic cell division, resulting in a two-cell haploid parthenogen. Johnson [5] reported that exposure to hyaluronidase or pronase did not cause activation among human oocytes. In addition, Abramczuk and Lopata [6] found that freshly retrieved human oocytes that were either cultured under IVF conditions or treated with hyaluronidase showed no signs of activation. These observations exclude oocyte pretreatment for ICSI as a possible cause of parthenogenesis.

In agreement with the current study, Muecheler *et al.* [7] concluded that the naturally occurring rate of parthenogenesis was extremely rare. Their results, however, included only one parthenogenetic oocyte which had two polar bodies and one nucleus. This particular oocyte had not divided and no analysis was performed to determine whether the cell was diploid or haploid. The current study is the first to document the unequivocal existence of naturally occurring spontaneous parthenogenesis while confirming the observations of the abnormal oocyte configurations through genetic assay. Nevertheless, the mechanism by which parthenogenesis occurs is still in serious need of elucidation. Further research should aim to understand how and why parthenogenesis occurs and what effects it may have on humans, particularly with regard to its correlation with ovarian teratoma.

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