Intrinsic fertility of human oocytes

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Objective: To study the intrinsic fertility of the human oocyte.
Design: A large retrospective study of natural cycle single embryo transfer (ET) IVF cycles.
Setting: Private IVF clinic, university, and private hospital.
Patient(s): Patients were enrolled consecutively over an 8-year period in a single ET natural cycle protocol.
Intervention(s): A total of 13,949 oocyte retrievals with natural IVF single ET. Software package R (version 3.2.5) was used for statistical calculations.
Main Outcome Measure(s): Live baby rate per oocyte according to age.
Result(s): A total of 14,185 natural cycle oocytes resulted in 1,913 live babies from single ET. The number of oocytes required to make one live baby in this large series varied with the age of the female partner. For those under 35, the live baby born per oocyte was 26%. For over age 42 it decreased to 1%. These results fit very robustly with a logistic function curve, which is at first steady (horizontal), followed by a linear decline after age 35 with a 10% loss every year until age 43, and then a flattening out (horizontal) by age 44.
Conclusion(s): The intrinsic fertility per oocyte in natural cycle is far greater than reported in hyperstimulated cycles, varying robustly from 26% to 4% with age from <35 to 42 years. The curve is relatively flat until age 34, and then declines rapidly 10% per year thereafter. (Fertil Steril® 2017;107:1232–7. ©2017 The Authors. Published by Elsevier Inc. on behalf of the American Society for Reproductive Medicine. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).)
Key Words: Intrinsic fertility, human oocyte, natural cycle, single embryo transfer, age

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The first successful human IVF was performed with natural cycle and single embryo transferred by Steptoe and Edwards in 1978 (1). Subsequently, ovarian hyperstimulation has been used to produce multiple oocytes and thus improve the pregnancy rate. It is generally accepted now that IVF requires ovarian hyperstimulation to produce many oocytes to increase pregnancy and live baby rate per egg retrieval (2–6). However, the pregnancy and live baby rate per egg rather than per cycle, that is, the average number of oocytes required to produce a baby, is a metric that in a natural cycle would give us the intrinsic fertility of the human egg without hormonal manipulation and without the confusion of statistically trying to account for untransferred frozen embryos (7, 8). Ovarian hyperstimulation in previous studies has been found to yield a live baby rate per oocyte of only about 4%–6%, and so on average more than 20–25 oocytes would be required to produce a single live baby, indicating an enormous oocyte wastage (7). However, there is a statistical problem with such a calculation. If there are untransferred remaining embryos (and there usually are), then it is impossible to determine the actual live baby rate per oocyte unless one "guesses" what the live baby rate would be from those untransferred embryos. Otherwise, the live baby rate per egg would be understated, and the number of eggs required to produce a baby would be overstated.

We originally wished to investigate the possibility of lessening IVF cost and morbidity with the recruitment of fewer oocytes, using natural cycles. That issue is still open to clinical debate. However, the main interest that evolved out of this study has become simply to answer the question, what is the baseline live baby rate per oocyte in an unstimulated cycle exclusively with single ET (the intrinsic fertility rate of the human egg), with no leftover frozen untransferred embryos? And by inference, what might be the limit of pregnancy expectation in any given month of unprotected intercourse?

MATERIALS AND METHODS

This retrospective cohort study involved routine consecutive nondonor natural

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IVF cycles at the Kato Ladies Clinic in Tokyo, Japan. The study was approved by the Institutional Review Board, and written informed consent was obtained from all patients. In a natural cycle single ET IVF program, 13,949 oocyte retrievals (ORs) yielded 14,819 oocytes (Table 1). Eliminating the occasional cycles with a leftover untransferred frozen embryo, a total of 14,185 oocytes were obtained from 13,386 cycles (Table 1). Note that even in a natural cycle, there will be occasional cases where more than one egg is retrieved "like in real life." All transfers were single embryo only. Results were divided into detailed subgroups by female age, whether fresh or frozen, and whether intracytoplasmic sperm injection (ICSI) or conventional IVF, detailing ET rate, cleavage rate, and blastocyst rate. There was no selection process. This was a uniformly Japanese population. Furthermore, pregnancy rates and live baby rates per egg were compared for blastocyst- versus cleavage-stage transfer and fresh versus frozen transfer, to determine whether there was any significant difference. This natural cycle protocol with single ET with no leftover frozen embryos would be appropriate as the best (if possible) estimate of the intrinsic fertility of the human oocyte. An oocyte was defined as a cumulus oocyte complex because in conventional IVF one does not determine whether it is a mature or immature oocyte.

To avoid any confusion for interpreting the results per oocyte, the 13,386 ORs involving 14,185 oocytes in which there were no remaining untransferred embryos were analyzed to determine the number of eggs required to produce a single pregnancy and a single live baby.

In our natural cycle protocol, the only pharmaceutical intervention was final oocyte maturation with a GnRH agonist (9, 10). Cycles were monitored by transvaginal ultrasonography, as well as measurements of serum E_2, LH, and P, which occurred from day 8 to 12. OR was scheduled when the leading follicle reached 18 mm in diameter with a concomitant serum E_2 level $\geq$250 pg/mL. Ovulation was triggered with the GnRH agonist busereline (600 μg; Suprecure, Aventis Pharma) administered in a nasal spray, followed by OR 32–35 hours later. OR was performed using a 21-gauge needle (Kitazato Medical). P supplementation was routinely used for all fresh and for all frozen ETs.

ICSI was performed when less than a total of 100,000 morphologically normal motile sperm were available post-wash. Fertilized two-pronucleate zygotes were cultured individually in 20 μL of cleavage-stage medium (Sage) for 2 or 3 days, and blastocyst culture was also performed using commercially available media (Quinn's Advantage; Sage). All the embryos were cultured at 37°C under a gas phase of 5% O_2, 5% CO_2, and 90% N_2 with full humidity in water jacket small multigas incubators (Astec). Blastocyst culture, elective vitrification, and subsequent frozen-thawed ET were performed routinely. Embryos that appeared to have good quality were transferred at cleavage stage. Others were cultured to blastocyst. If a patient had poor endometrium, or slowly developing blastocysts, or if there was simply a scheduling preference, the embryos or blastocysts were cryopreserved. Whether it was a cleavage-stage transfer or blastocyst, only one embryo was transferred. A single embryo was transferred, and only cycles in which there were no remaining frozen embryos are included in the calculations of pregnancies and live baby rate per oocyte. Since this was natural cycle, almost all cycles involved only one egg and one embryo, although a very small number yielded two oocytes and two embryos, and they were excluded, as mentioned previously.

TABLE 1

Oocyte retrievals in natural cycles.

Age group (y)	Patients	OR cycles	Oocytes	Oocytes per OR
A. Before subtraction of patients with leftover frozen embryos				
<35 (21–34)	3,457	2,911	3,075	1.1 ± 0.3
35–37	3,726	3,115	3,269	1.0 ± 0.2
38–40	2,788	2,519	2,647	1.1 ± 0.2
41–42	1,652	1,737	1,865	1.1 ± 0.3
>42 (43–54)	2,400	3,667	3,963	1.1 ± 0.3
Total ≤42	11,623	10,282	10,856	1.1 ± 0.3
Total	14,023	13,949	14,819	1.1 ± 0.3
B. After subtraction of patients with leftover frozen embryos				
<35 (21–34)	2,582	2,860	2,991	1.1 ± 0.3
35–37	2,810	3,030	3,176	1.0 ± 0.2
38–40	2,035	2,334	2,449	1.1 ± 0.2
41–2	1,261	1,650	1,772	1.1 ± 0.3
>42 (43–54)	1,731	3,512	3,797	1.1 ± 0.3
Total ≤42	8,688	9,874	10,388	1.1 ± 0.3
Total	10,419	13,386	14,185	1.1 ± 0.3

Note: Values presented as n or mean ± SD, unless stated otherwise. OR = oocyte retrievals.

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Embryo/Blastocyst Vitrification, Thawing Protocol, and ET

All embryos and blastocysts that were cryopreserved used the Cryotop vitrification method as described elsewhere (Kitazato Medical) (9–12). Thawing of the vitrified embryo consisted of an ultraquick warming in a 37°C thawing solution, and then cryoprotectants were completely diluted in washing steps; 99% of the cryopreserved embryos survived the thaw. Both fresh and frozen transfers were used to determine total live births per ET and per oocyte. A total of 6,983 transfers were performed. The majority of transfers were fresh (5,833 vs. 1,150), and the division between ICSI and conventional IVF was 4,006 ICSI, versus 2,977 conventional IVF.

Statistical Analysis

The primary data of live baby per oocyte were approximated with a logistic curve $r = 1/(a + \exp[b(t - c)])$, where r is live baby rate per oocyte and t is age in years. The coefficients were evaluated using the gradient method as implemented in statistical package R (ver. 3.2.5). The secondary data of pregnancy and live baby rate per transfer using ICSI or conventional IVF or fresh versus frozen transfer were analyzed with the chi-square test. It was important to evaluate whether there were differences between fresh or frozen transfer, between cleavage-stage transfer or blastocyst transfer, and between pregnancy and live baby rate per oocyte according to age. Very few patients underwent more than one (OR) cycle (1.3 OR cycles per patient), because if they failed natural cycle, they would usually undergo mild stimulation IVF for the subsequent cycle.