Automated Cell Division Detection and Classification in Early Mouse and Human Embryos

by
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We hereby certify that this dissertation, submitted by Jonas Malmsten, satisfies the dissertation requirements for the degree of *Doctor of Professional Studies in Computing* and has been approved.

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Abstract

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Infertility affects millions of couples every year. The most effective treatment for infertility is in-vitro fertilization (IVF). Embryo selection, which has become a critical part of IVF treatments, is the process of selecting the most viable embryos to transfer and maximize the chance of pregnancy while minimizing the risks resulting from multiple births. Advancements in time-lapse microscopy have provided a stepping stone for better assessments of embryo quality but have also placed a greater burden on embryologists having to review a steadily growing number of images. Recent developments in artificial intelligence and convolutional neural networks (CNN) have shown great potential in computer-aided analysis of images. In this work, we explore ways to use CNNs to automate the embryo selection. In particular, we focus on predicting the timings of cell divisions in early human embryos, which have been shown to correlate well with pregnancy outcomes. In conclusion, we present a method to aid embryologists with annotating embryos and in a future version we think our method will be a part of a fully automated system for selecting the most viable embryos.

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Chapter 1

Introduction

1.1 In Vitro Fertilization

Robert Edwards and Patrick Steptoe invented and performed the first successful in-vitro fertilization (IVF) procedure in 1978 [1]. In vitro means in glass, or in general outside of the living organism. Oocytes (unfertilized eggs) are surgically retrieved from a patient's ovaries, fertilized in vitro (union of female and male gametes), and transferred back to the uterus to achieve pregnancy. Infertility is often caused by problems with the uterus or fallopian tubes, preventing sperm from reaching the oocyte. The IVF procedure directly circumvents these and, as we shall see, many other problems as well. Robert Edwards received the Nobel Prize for his achievements in 2010.

Assisted reproductive technology (ART, including IVF) is a very controversial topic with ethical concerns, from both political and religious views, and whether we should tamper with the creation of life. Despite the controversy, the market for ART has grown continuously since inception. It is no longer unusual for people to seek professional help with infertility and ART has become more socially accepted. IVF became available to patients in the US in 1981 in Norfolk, VA. Society for Assisted Reproductive Technology (SART) was founded in 1985 to provide guidelines, represent, and promote the ART community, in which they have been very successful. Since 1992 all ART procedures should be reported to the Center for Disease Control and Prevention (CDC), who publish

yearly national statistics. In 2015, the US fertility market was estimated to be between $3 and $4 billion [2], and there were 231,936 ART procedures performed using IVF resulting in 60,778 live births and 72,913 children [3]. With 3,978,497 births reported in the same period, 1.8% of all children born in the US were from IVF. There is no indication that the trend of continuous growth is about to change, Table 1.

Table 1 National ART Statistics

Year	Total Children Born in the US	ART Cycles	Births from ART	Children from ART	%Twin Births from ART	%Children from ART
2015	3,978,497	231,936	60,778	72,913	20.0	1.83
2014	3,988,076	208,786	57,332	70,352	22.7	1.76
2013	3,932,181	190,773	54,323	67,996	25.2	1.73
2012	3,952,841	176,247	51,267	65,160	27.1	1.65
2011	3,953,590	151,923	47,818	61,610	28.8	1.56
2010	3,999,386	147,260	47,090	61,564	30.7	1.54
2009	4,130,665	146,244	45,870	60,190	31.2	1.46
2008	4,247,694	148,055	46,326	61,426	32.6	1.45
2007	4,316,233	142,435	43,412	57,569	32.6	1.33
2006	4,265,555	138,198	41,343	54,656	32.2	1.28
2005	4,138,349	134,260	38,910	52,041	33.7	1.26

1.2 The IVF Treatment

There are many variations of how to perform an IVF treatment. Table 2 provides an overview of the most common steps. For each treatment, there is a time-line starting with day 1 of the patient's menstrual cycle. The average length of the cycle is 28 days. Embryo selection, which is the main topic of this study, is done during the 3-5 day incubation period starting usually on day 16. The days indicated are average numbers and may vary slightly from one treatment to another. For example, the length of the follicular phase is determined based on the results of sonograms (ultrasound) and blood tests. If HCG trigger (ovulation

trigger) is injected on day 12 instead of 14, retrieval will be on day 14 and transfer will be on day 17 or 19.

The first pregnancy test is performed on, or after, day 28. The first sign of a successful pregnancy is a positive βHCG test; an elevated level of beta-human chorionic gonadotropin (βHCG) in the bloodstream. A clinical pregnancy is established by observing sacs or fetal hearts during a sonogram, and may not be visible as early as day 28. A visible sac in the uterus is proof of implantation; that the embryo has implanted in the uterus wall. A heartbeat should be visible soon after, at which point the patient is usually done at the IVF clinic and referred to a regular OB/GYN clinic to follow-up on their pregnancy. When measuring the number of successful pregnancies, the implantation rate is sometimes used instead of actual live births. In part because live birth data is not always available, but also because there are many non-embryo related events that can cause a pregnancy to fail after implantation.

Table 2 Overview of an IVF treatment

Day	Short Name	Description
1		The first day of bleeding.
2-3	Start Stims	Start hormone injections to stimulate follicles to produce more oocytes. Injections continue daily for about 12 days.
2-14	Follicular Phase	Sonogram and blood tests are used several times to monitor the development of follicles in the ovaries. Each follicle contains one oocyte and grows in size as the oocyte matures.
14	HCG Trigger	Human chorionic gonadotrophin (HCG) is injected to trigger ovulation; the release of oocytes from the ovaries into the fallopian tubes where natural fertilization would occur. Day 14 is an average; the previous monitoring determines the exact day. HCG will induce ovulation;
16	Retrieval	36 hours after HCG trigger a surgical procedure is used to retrieve the oocytes from the follicles just before ovulation.
16	Fertilization	Retrieved oocytes are fertilized with sperm and placed in an incubator. They remain in the incubator for 3-5 days.
19 or 21	Transfer	The embryos (fertilized oocytes) are transferred back to the uterus to implant and achieve pregnancy.
28		The first pregnancy test.

1.3 Advances in ART

The technology used to perform IVF has evolved significantly over the past decades [4], resulting in both increased success rates and the ability to treat different problems. Patients chose IVF for various reasons. The most common reason is that they are unable to achieve pregnancy on their own within a certain period of trying. Advances in the surgical procedure to retrieve oocytes and the use of stimulated cycles have had a huge impact on IVF success rates. During a regular menstrual cycle, the ovaries release on average one oocyte each month. The number of oocytes retrieved in the original natural (non-stimulated) cycles from 1978 was only about 0.7 with each attempt [4]. In a modern stimulated cycle, the patient injects daily hormones for about two weeks before retrieval increasing the number of oocytes tenfold; retrieval of 10-20 oocytes is common. After

fertilizing all oocytes, creating embryos, only a few of them are selected for transfer back to the patient. The process of selecting the most viable embryos for transfer is referred to as embryo selection and has become a critical part of IVF that will be explained further in the next section.

Cryopreservation is a technology that has opened up for new reasons to perform IVF, as well as improved safety and efficiency of regular IVF. Oocytes, sperm, and embryos can be cryopreserved (frozen) and kept indefinitely before they are thawed and used. Fertility preservation (FP) is a process when patients chose IVF just for cryopreservation without intent to become pregnant. Some chose it for social reasons, like delay having children. Others have medical reasons, for example, cancer patients who know they will become infertile after cancer treatment.

Elective single embryo transfer (eSET) is a way to reduce cost and risk when doing IVF with the intent to become pregnant. eSET was made available by advancements in embryo cultures, cryopreservation and the general increase in IVF success rates. During a traditional IVF cycle, it is common to transfer two or more embryos to increase the chance of success on the first attempt. However, this also increases the chance of multiple pregnancies (twins and triplets). Multiple pregnancies have an increased risk of complications for both mother and children. There is an increased rate of early births resulting in longer hospital stays and higher costs. The idea behind eSET is to transfer only one embryo from a fresh IVF cycle and cryopreserve remaining embryos. If the IVF cycle fails, another attempt is made using a frozen embryo transfer (FET). FET is cheaper and less invasive because there is no need for hormone stimulants or oocyte retrieval. The

benefits of not having twins have been shown to outweigh the cost of performing the extra procedure [5]. Embryo selection becomes even more important with eSET.

Advances in preimplantation genetic testing (PGT) can be used to de-select unwanted abnormal embryos. A small sample, or biopsy, is taken from each developing embryo and sent to a genetics laboratory. The laboratory can detect genetic mutations or chromosomal abnormalities, like aneuploidy, which often results in miscarriages. DNA can be sequenced and analyzed for inheritable diseases. PGT can also be used to select gender and other traits. Deselecting embryos to eliminate well-documented diseases is more common, and more socially and ethically acceptable, than selecting embryos for particular traits [6].

Male infertility is about as common as female infertility [7]. Female infertility often results from problems with ovaries, fallopian tubes, or hormonal imbalance, which are inherently solved by the IVF procedure. Male infertility may result from low sperm count or poor sperm motility, which can be treated using intracytoplasmic sperm injection (ICSI) [8]. ICSI means using a fine needle to inject one spermatozoon (5μm) into each oocyte. Other procedures for male infertility include testicular sperm extraction (TESE).

Donation programs have expanded the market for ART further. Patients who are medically unable to produce oocytes or sperm can use a donation program. Donation programs also allow for singles or same-sex couples to become parents.

1.4 Embryo Development

After retrieval, the oocytes are fertilized with sperm and placed in an incubator providing a suitable environment for the embryos to develop. Temperature, pH, and gas levels are

regulated while minimizing exposure to light. The embryos remain in the incubator for 3-5 days before they are ready to be transferred back to the patient. During this time they are monitored closely. It is common to observe embryos through a microscope to assess embryo quality at regular intervals. However, with the development of the time-lapse microscopy (TLM) embryos can be monitored continuously without disturbance. Figure 1 illustrates some of these TLM images captured at different stages of embryo development. The first image is a zygote, that is an oocyte immediately after fertilization. There is no visual difference between an oocyte and a zygote until several hours later. A zygote consists of one cell surrounded by a thin shell called the zona pellucida (ZP). One of the early indications of successful fertilization is the appearance of 2 pronuclei (2PN, male-sperm and female-oocyte origin) as shown in the second image. The pronuclei are the small circular regions slightly above the center. These are the male and female chromosomes about to combine. Once combined, the cell starts to divide to form 2, 4, and 8 -cell embryos (cells called blastomeres), referred to as cell stages. Cell stages 3, 5, 6, and 7 are also possible, but usually for a much shorter duration. Blastomeres become too small to identify after 8-cell stage. As depicted in the last image, after about five days, the embryo forms a blastocyst. A blastocyst consists of three main parts: Trophectoderm (TE), inner cell mass (ICM), and the blastocoele. The TE is the surrounding shell of the embryo that will form placenta, the ICM is the lump of cells seen towards the left of the center of the image that will form fetus, and the blastocoele is the fluid-filled cavity between the ICM and the TE.

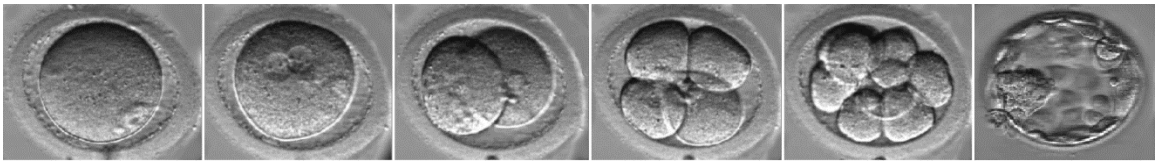

Figure 1 A normal developing embryo at different cell stages with mean hours since fertilization (based on our dataset).

1.5 Embryo Selection

Embryo selection is the process of selecting which embryos to transfer back to the patient, and its impact on IVF success rates is well documented [9-11]. Embryos are sometimes transferred back to the patient after three days of incubation, at around the 8-cell stage. Other times they are left in the incubator for about five days and transferred back to the patient as blastocysts. When the embryos are transferred is usually decided by the embryologist and physician and is determined by embryo number and quality. Which embryos to transfer is usually determined by an embryologist observing several indicators of embryo quality at various cell stages. The observations are combined into an embryo grade, explained in the next section. Many miscarriages and failed pregnancies are due to genetic defects. There is a correlation between certain genetic defects and the timing of blastomere divisions seen as early as the 8-cell stage [12]. The objective of this research is to develop a method for automatic detection of blastomere division events in a series of digital images of developing embryos.

1.6 Embryo Grading

The goal of embryo grading is to assign a score to each embryo, such that the scores are highly correlated with positive pregnancy outcomes [13]. A positive pregnancy outcome can be live birth or that the embryo implanted. Many things can go wrong between implantation and live birth, and these problems are often not related to embryo quality. Grading is done just before transfer, after three or five days of incubation. The grading on day three is quite different from that of a day five. After three days, the embryo is expected to have around eight blastomeres. A score between 1 (good) and 5 (poor) is assigned by an embryologist based on visual inspection of the embryo. The embryologist typically looks for features such as the number of blastomeres, their sizes and symmetry, fragmentation (cytoplasmic blebs), blastomere lineage and timing of cell division events, ZP thickness [14], zygote and pronuclear morphology [15]. A day five embryo is expected to have formed a blastocyst. Many clinics follow the Gardner blastocyst grading system [16], where individual scores are assigned for expansion, ICM, and TE. The expansion grade is assigned a number between 1 and 6, ICM and TE are both graded with letters A, B, or C, where 3, 4, or 5AA is the best possible grade. Figure 2 illustrates some of the observed features.