Lecture Summary

From ovary to stock exchange - egg cells, stem cells, therapies and women on a global health market

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1 Human egg cells

Primary oocytes (egg cells) are formed in the female fetus in primary follicles within the ovaries. At week 20 of the fetal development, the ovary contains about 7 millions of oocytes. This number is reduced to about 2 million oocytes at birth by a process called follicle atresia. Due to rising estrogens in puberty, oocyte numbers are reduced to about 250.000 and further decline with obtaining a regular menstrual cycle and then over the years until oocyte maturation in follicles ceases completely during menopause. Naturally, about 300 to 500 mature oocytes are formed during a woman's reproductive years. In analogy to sperm production (after puberty, about 100 million sperm cells are produced per day), it is often argued that a woman has enough oocytes and therefore it does not matter if she is giving away some of them. However, considering the above mentioned oocyte counts puts this analogy into perspective and the fact that the major part of a woman's prenatally formed gametes degenerates and only a minor proportion matures to a stage allowing for fertilization underlines that oocytes are in fact a very limited resource. Moreover, formation of mature oocytes is a sophisticated biological process influenced by a number of factors. Oocytes of different maturation states are found in follicles throughout life and even in the newborn baby, some mature oocytes can be formed due to maternal estrogens.

Whereas obtaining sperm does not require any pharmacological intervention or invasive procedure (except for those clinical conditions of male factor infertility, in which no or only non-viable sperm cells are present in the ejaculate), obtaining oocytes does. The process of oocyte retrieval developed for *in vitro fertilization* (IVF) technologies, which is often termed "egg collection" or "egg harvest" thus stating clearly what the process means,

comprises (1) the hormonal suppression of the endogenous production of follicle-stimulating hormone, (2) the hormonal stimulation of ovaries to induce follicle maturation, (3) a final egg maturation-inducing step, and (4) a surgical intervention to aspirate mature follicles from the ovaries, usually by transvaginal puncture. Depending on the protocols used, the procedure takes 2 to 4 weeks, requires daily hormonal injections (depending on protocol up to 40) and numerous blood and ultrasound tests and ideally should be adapted to an individual woman's needs defined by her health status, her age, her hormonal status and her reaction to the drugs applied in order to minimize the risk for adverse reactions, i.e. developing ovarian hyperstimulation syndrome (OHSS). OHSS can occur in various stages from clinically irrelevant in about 30 % of women to life-threatening conditions (caused by edema, increased blood viscosity, ascites and renal failure) in about 5 % of women. There are a number of risk factors for developing OHSS, e.g. polycystic ovary syndrome (the leading cause for impaired fertility; the condition is correlated to insulin resistance, diabetes and obesity), elevated testosterone levels, young age, underweight, high estradiol levels, pituitary adenoma (tumors of the pituitary gland which often remain undiagnosed but start to grow upon progesterone during pregnancy or upon hormone stimulation), or application of high hormone doses in order to stimulate maturation of numerous follicles for IVF or egg cell donation. In some of these conditions, severe OHSS can occur in up to 38 % of women. Due to these known risks, "mild" or "natural cycle" protocols are developed and favoured by numerous experts, e.g. also by R. Edwards who was responsible for the first successful IVF leading to the birth of Louise Brown in 1978.

In addition to OHSS, women undergoing egg cell maturation protocols are at risk to develop infections and ovarian cysts that could impair their fertility. Moreover, an increased risk to develop breast and ovarian cancer is discussed and there are anecdotal reports of advanced and incurable, rapidly growing colon cancer in young women after ovarian stimulation. One major point of concern is that so far no systematic studies concerning long-term effects of repeated ovarian stimulation have been performed. In the context of egg cell donation, the lack of a registry of the donors is critical. In addition, when it comes to safety of egg cell donation, the FDA (Food and Drug Administration, USA) guidelines for stem cells deals exclusively with product safety, i.e. the safety of cell lines resulting from using donated egg cells, but not with safety for egg cell donors.

Taken together it is clear that stimulating the maturation of several egg cells, either for egg cell donation or in the process of IVF, is a cumbersome procedure with a number of risks that are generally played down but in the case of OHSS not at all rare.

2 Who needs egg cells?

Egg cells are required by IVF patients whose own egg cells cannot be used because of e.g. early menopause, malfunctioning ovaries, unsuccessful IVF with own egg cells, increased risk for inheritied disease or age. Another "user" for human egg cells is embryonic stem cell research. In general, embryonic stem cells are established from IVF embryos that are not used for IVF. The vision of creating patient-specific embryonic stem cell lines by a method called *somatic cell nuclear transfer* (SCNT) or cloning boosted this research branch leading to a need for egg cells by stem cell research. However, so far no cloned human embryonic stem cell lines could be established but only 5 blastocysts (early-stage embryos) were established by cloning and resulted from 29 egg cells taken from egg cell donations of young healthy women donating for IVF[1]. In addition, egg cells can also be triggered in culture to develop into embryos by a process called parthenogenesis. This does not require fertilization or cloning[2]. Cell lines can then be established from these parthenotes. Also these protocols have only a low efficiency in giving rise to stem cell lines, e.g. from 44 donated egg cells, 23 blastocysts could be generated by parthenogenesis, from which 6 embryonic stem cell lines could be established[3].

In both applications, human egg cells have the potential to offer something very individual, in one case the "own" child, in the other case the "personalized" cell line. Particularly the latter is still highly experimental.

3 Embryonic stem cells (hESC)

hESCs are characterized by the potential for indefinite division and to develop into all known cell lineages. The vision of regenerative medicine is to use stem cell transplants in order to cure diseases caused by tissue damage. To limit adverse immune reactions to such transplants, creation of patient-specific cell lines is a major goal of research. This is achieved by cloning established so far only for animal models, e.g. mice and primates. Therefore, the currently

[1] French et al., *Stem Cells* 26, 485-493, 2008

[2] Cloning means that the egg cell's nucleus is removed. Then, the cell nucleus from an adult body cell is introduced into the enucleated egg cell. The cellular machinery of the egg cell then "reprograms" this adult cell nucleus back into an embryonic stage allowing this artificial entitiy to develop into an embryo. From this embryo, a stem cell line can be established which is genetically a clone of the donor of the adult cell nucleus. This donor could be a patient. The resulting cell line would be immunologically identical and could therefore be used as a transplant and no immunosuppression would be required.

[3] Revazova et al., *Cloning Stem Cells* 9, 432–449, 2007

available hESC lines are not cloned, i.e. there application as transplants would require immunosuppression.

The first 5 hESC cell lines were established some 10 years ago[4] and are still widely used for research although they were grown on mouse-feeder cells and in presence of animal serum[5]. Since then, a number of stem cell banks have been established (e.g. the NIH Human Embryonic Stem Cell Registry (now called Human Pluripotent Stem Cell Registry, banking the "presidential lines", i.e. hESC established before 2001 when former US-president Bush stopped national funding for new hESC lines; WiCell (Univ. of Wisconsin, Madison) – National Stem Cell Bank (since 2005); European Human Embryonic Stem Cell Registry (EC-funded, 2007, Barcelona/Berlin); UK Stem Cell Bank). Taken together, around 250 hESC lines are available from these institutions. However, the number of non-registered hESC lines appears to be much higher[6].

Despite major efforts in hESC research, the first Phase I clinical trial world-wide was permitted only in January 2009. Neuronal precursor cells derived from hESC will be used for injecting 8-10 patients suffering from acute spinal cord injury. The primary endpoint of this trial is safety and it is hoped that also some results on clinical efficacy can be obtained[7]. The trial is criticized for relying on a doubtful rational and for not relying on the best first candidate for a clinical trial[8].

Although so far there is no such thing as embryonic stem cell therapy, the term "therapeutic cloning" was coined already several years ago over the hype on the work by the Korean stem cell researcher Hwang who had claimed that he had cloned patient-specific cell lines. Already before Hwang's work turned out to be one of science's major fraud cases and had to be retracted in the beginning of 2006, bioethicists criticized that„the language used to describe the research can reinforce the therapeutic misconception, misleading donors and subjects into believing that research is therapy"[9]. Moreover, already at this time when the vision of patient-specific hESC seemed to become true, it was warned that healthy women should not donate egg cells for this research because of the risks of the procedure and no benefit for the donors[10]. Nevertheless, only after the Hwang scandal, finally the question arose where the egg cells for this research were coming from. It turned out that for the two retracted

[4] Cell lines H1, H13, H14 (male) and H7, H9 (female), Thomson JA et al., *Science* 282, 1145-1147, 1998
[5] Both culture conditions are major draw-backs for therapeutic application because cell lines express animal antigens on their surface and might take up non-identified animal viruses that could become pathogenic when entering a new host such as a human. Therfore, these cells have to be purified by elaborate procedures.
[6] Scott CT, *Neurosurg Focus* 24, 1-4, 2008
[7] http://www.geron.com/patients/clinicaltrials/hESC.aspx
[8] Wadman M, *Nature* 457, 516, 2009; Couzin J, Science 323, 568, 2009
[9] Magnus & Cho, *Science* 308, 1747-78, 2005
[10] Magnus & Cho, *Science* 308, 1747-78, 2005

papers, in which the faked data were published, about 2200 egg cells from 119 women had been used. This means that on average, some 18 egg cells were obtained from each donor, indicating that the stimulation protocols used were optimized for obtaining many egg cells and not for minimizing risk. Accordingly, 15 to 20 % of the women developed severe OHSS[11]. This case made it finally clear that the availability of human egg cells is one of the major limitations to develop this method. In addition, the whole field is controversial in many countries because of destroying viable human embryos.

Recently, a new technology which avoids using egg cells or destroying embryos was established, i.e. the production of so-called *induced pluripotent stem cells* (iPSC). These are adult cells, usually fibroblasts from the skin, which are manipulated to "reprogram" to a less differentiated state. By this method, pluripotent cells could be developed, this means cells with the quality to differentiate into all known cell lineages like hESC. The first patient-specific cell lines (from patients with neurodegenerative disease) could already be developed. Moreover, it might be possible to avoid tumor-formation, a major drawback for the clinical application of pluripotent cells[12]. Currently, a lot of progress is made in this field, because many groups can perform this kind of work whereas research on hESC is strongly regulated and licensed only to comparatively few laboratories world-wide. Interestingly, most leading hESC researchers have switched to iPSC and think that these cells have a much better clinical potential than hESC. For example, James Thomson, the first one to establish hESC lines, anticipates that most scientists „will migrate over to iPS cells since they don't have all of the baggage that ESC's do". He further thinks that the „long-term goal of regenerative medicine is to cause tissue to regenerate, not to do cell transplantation". He anticipates that „there'll be a lot of failures" regarding stem cell therapy[13]. Thomson's statement indicates that one of the most eminent stem cell researchers considers the current concepts of therapeutic cloning and the production of stem cell transplants to be inadequate for resolving the clinical questions for which they were developed.

Against this background, the ongoing lamentation of stem cell researchers regarding the "shortage" of egg cells for their research is highly questionable. A major reason for this "shortage" seems to be the fact that no or only a minimal compensation for egg donation for research is allowed in most countries and US-states[14] while in IVF, financial compensation is standard. Eggan and Melton, two stem cell researchers from Harvard, reported that it took

[11] A comprehensive summary of egg cell "donation" in the Hwang case is found in Dickenson, D., Body Shopping, Oneworld Publications 2008, chapter 4.
[12] Reviewed in Yamanaka S, *Cell* 137, 13-17, 2009
[13] Gitschier J, *PLoS Genetics* 4, 1-3, 2008; Stojkovic M/Daher S, *Stem Cells* 26, 2747-2748, 2008
[14] More details in Steinbrook R, *N Engl J Med* 354, 324-326, 2006

them 2 years and 100.000 USD for local advertising in order to recruit a single egg donor of who they retrieved less than 10 egg cells[15]. Other researchers ask for a compensation between 3.000 to 5.000 USD for donation because of the arduous procedure and D. Batzofin, the administrator for La Jolla IVF, a fertility clinic, states: "I don't see how they would expect any woman to do it without getting paid for it"[16]. Interestingly, while it is always claimed in the IVF context that egg donation is an unproblematic procedure and that the payment is just a compensation for expenses, stem cell researchers emphasize the arduousness of the procedure which would ask for compensation. In line with this, the state of New York is working on new guidelines allowing to pay for egg cells donated to research in order to support stem cell research and to attract researchers to move there[17].

The scarcity of egg cells leads also to close cooperation of stem cell researchers with IVF clinics. "Egg sharing" programmes, e.g. in England, allow women to obtain cheaper IVF if they give some of their egg cells to research. This is highly controversial because poorer women might be exploited, protocols optimized for obtaining many egg cells instead of less stressful protocols are necessary in order to get enough cells and the quality of egg cells from an IVF patient may not be good enough for cloning experiments which was so far successful only with fresh, high quality egg cells[18]. In addition, the connection between IVF and stem cell research generates a market of "reproductive work" for stem cell research[19].

In order to regulate egg cell donation for research, it was claimed that egg cell donors should be considered to be organ donors and it was also criticized that informed consent might focus too much on research aspects rather than on health risks. This might lead to a misunderstanding on the current status of research, thus leading women to donate cells in order to help a loved one while not knowing that the current technology is still far away from therapy[20].

4 Marketing of cells, therapies and women

With regard to egg cell donation, different regulations exist within the EU. Whereas egg cell donation or import is illegal in Austria and Germany, it is allowed in several European countries in the context of IVF. Usually, donors should be young, healthy and educated, in

[15] Maher B, *Nature* 453, 828, 2008
[16] Bennet D, voiceofsandiego.org, Aug 7, 2008
[17] Bennet D, voiceofsandiego.org, Aug 7, 2008
[18] French et al., *Stem Cells* 26, 485-493, 2008
[19] Waldby C, *New Genetics and Society* 27, 19-31, 2008
[20] Levine D, *San Francisco Business Times*, Feb 3, 2006; Egg extraction for stem cell research: issues of women's health, http://www.geneticsandsociety.org/article.php?id=950 (accessed on June 22, 2009)

Russia they should also have already own children. In England, IVF and egg donation is regulated by the Human Fertility and Embryology Authority (HFEA). Since 2007, a compensation of 250 £ can be given for donated eggs, the above-mentioned possibility of "egg-sharing" exists and egg cells may also be donated for research which is prohibited in many other countries. In Spain, egg donation is unregulated and compensations of about 1.000 Euro are common. Spain is the main exporter of egg cells in Europe. Since 20 years, building a gamete registry is legally required but this law was never put into practice, a situation preferred by the Spanish IVF industry[21].

The different regulations create a market for egg donation and "IVF-tourism", particularly between central European and Southern- and Eastern-European countries. After the public got notice of Romanian women who sold their egg cells for a compensation of 250 USD to British couples (who had costs of around 8.000 USD) via the GlobalART clinic in Bucharest in 2004 and that women who developed OHSS got no treatment by the clinic, the European Parliament adopted a resolution on the trade in human cells (March 10, 2005)[22]. Nevertheless, it seems that the market is flourishing, sometimes causing malpractice of criminal dimensions, as in the case where up to 70 egg cells were retrieved in one cycle, causing, as could be expected, severe OHSS[23].

In the US, it is estimated that the "IVF-industry" is an about 3 billion USD/year business[24]. Unlike in Europe, donors are not anonymous. Prospective parents or donors can make use of „egg brokers", „egg donation programmes", and „egg agencies" maintaining data banks freely accessible via the internet listing ethnicity, height, eye colour, education etc.[25]. Newspaper ads like „donor ideally has artistic skills as intended mother is a talented oil painter and piano player" can be found and up to 100.000 USD are offered via internet or newspapers for egg cells from "Ivy League" donors[26]. In general, academic medical institutions offer payments between 5.000 to 10.000 USD, which is in line with the guidelines of the ethic's committee of the American Society for Reproductive Medicine, that stated that more than 5.000 USD should be justified and more than 10.000 USD are considered to be unethical[27].

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[21] according to I. Alkorta Idiakez, http://www.boell.de/alt/downloads_uk/AlkortaIdiakez_Workshop2a.pdf, accessed on May 12, 2009

[22] http://www.europarl.europa.eu/sides/getDoc.do?pubRef=-//EP//TEXT+TA+P6-TA-2005-0074+0+DO, accessed on May 12, 2009

[23] Jacobs A., *Hastings Center Report* 31, 12-14, 2001

[24] Spar D*, N Engl J Med* 356, 1289-1291, 2006

[25] e.g. Egg Donation Inc. – *Where Dreams Come True,* http://www.eggdonor.com, accessed on May 12, 2009

[26] Hopkins J*, USA Today online,* March 15, 2006

[27] ECASRM, *Fertility and Sterility* 88, 305-309, 2007

Interestingly, suggestions about how often a woman can undergo follicle stimulation protocols, whether in the course of IVF or as a donor for IVF or stem cell research, vary considerably: e.g. many US-IVF centers advise that individuals should not donate more than 6 times[28], whereas an Israeli IVF clinic states on its homepage "Medically speaking, IVF treatment can be repeated every 2 months, and, in special cases, every month" so that "a total of 5-7 treatments per year" can be carried out[29]. In contrast, the "egg donor protocol and risks" document of the Bedford Stem Cell Research Foundation states: "OHSS can be avoided entirely by administering relatively low doses of gonadotropins and carefully measuring the estradiol response in the blood stream. At the first sign of an over-response, discontinuing the gonadotropins will eliminate the risk. The long-term risks of taking increased levels of gonadotropins will not be known for another decade or two, but are thought to be low. Risks are further limited by not undergoing more than 2 to 3 cycles of egg collection"[30]. This variation between 2 to 3 cycles in life to 7 treatments per year reflects the different interests of stem cell research foundations and IVF clinics. It also indicates that so far the stem cell business is comparatively less thriving as compared to the IVF business.

However, it was expected that rescinding the restrictions of federal funding for human embryonic stem cell research established by the Bush administration in 2001 by Obama's executive order signed on March 9, 2009, would trigger the stem cell business. Although, federal funding will still not be available for the derivation of new stem cell lines from discarded human embryos nor for parthenote cell lines, the shares of numerous stem cell companies rose considerably already in response to this expectation[31]. The author of this report admits that technically, all of these stocks are speculative stocks in the stem cell sector. This underlines that in the stem cell field, women's egg cells are used to foster a highly speculative industry that is marketing to-be therapies (analogous to the IVF-industry where to-be children are marketed along with egg cells) on a global market. In this context, a report about the International Stem Cell Corp. (ISCo) should make us prick our ears: it is stated here that directly stimulated egg cells (i.e. parthenotes) have the advantage that there is no embryo that could develop into a child (an "ethical" consideration) and that there are less complications to expect because such cells stem only from one parent and are therefore "immunologically simpler" (a scientifically flawed, but therapy-friendly consideration).

[28] Hopkins J*, USA Today online,* March 15, 2006
[29] Assuta Hospital IVF Center, Israel; http://www.ivfisrael.co.il/showArticle.asp?id=276&categoryId=86, accessed on June 22, 2009
[30] http://bedfordresearch.org, accessed May 11, 2009
[31] Ogg JC, Jan 23, 2009, http://www.biohealthinvestor.com/tag/embryonic-stem-cells, accessed on May 12, 2009

Hence, “replacement stem cells might then be ordered off the shelf”[32]. Thus it appears, that even although there is so far no established embryonic stem cell therapy, and despite that major concepts of such therapies are currently reconsidered and iPSC might open a completely different way to develop therapies, a growing industry branch is already relying on “products” from women’s egg cells.

[32] The Economist, Jan 19, 2009, http://www.economist.com/science/displaystory.cfm?story_id=13014104, accessed on May 12, 2009