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Success after failure: the role of endometrial stem cells in recurrent miscarriage

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Abstract

Endometrial stem-like cells, including mesenchymal stem cells (MSCs) and epithelial progenitor cells, are essential for cyclic regeneration of the endometrium following menstrual shedding. Emerging evidence indicates that endometrial MSCs (eMSCs) constitute a dynamic population of cells that enables the endometrium to adapt in response to a failed pregnancy. Recurrent miscarriage is associated with relative depletion of endometrial eMSCs, which not only curtails the intrinsic ability of the endometrium to adapt to reproductive failure but also compromises endometrial decidualization, an obligatory transformation process for embryo implantation. These novel findings should pave the way for more effective screening of women at risk of pregnancy failure prior to conception.

Introduction

Successful implantation of a human embryo is commonly attributed to binary variables; i.e. nidation of a 'normal' but not 'abnormal' embryo in a 'receptive' but not a 'non-receptive' endometrium is required for a successful pregnancy. However, this implantation paradigm is based on animal studies, more specifically the mouse model (Wang and Dey, 2006). Like many other rodents, mouse reproductive success is based on quantity: characterized by rapid breeding cycles, multiple synchronous implantations, large litter size, and, crucially, huge natural selection among offspring (Taylor, 2016). Mouse offspring "quality" is arrived at mainly through sibling rivalry after birth. By contrast, human pregnancy requires prolonged investment in a single fetus at considerable cost to the mother (Haig, 1993). Maternal cost, and the risk of neonatal death, increases sharply with each additional fetus (e.g. twins, triplets etc.) (Refuerzo, 2012). From an evolutionary perspective, a reproductive strategy based on prolonged maternal investment in singleton pregnancies makes sense only if based on an intrinsically dynamic and adaptable implantation process (Macklon and Brosens, 2014).

And it is. Cleavage-stage human embryos both tolerate and actively generate aneuploid blastomeres through mitotic non-disjunction. As a consequence, most human embryos are mosaic (Vanneste et al., 2009, Taylor et al., 2014). With over 2,500 distinct genetic errors documented to date (Fragouli et al., 2013), each implanting blastocyst is arguably unique. Furthermore, transient aneuploidy during development may not be unequivocally as 'bad' as has been intuitively presumed because of the obvious link with cancer and congenital abnormalities. Emerging evidence suggests that aneuploidy drives rapid phenotypic adaptation (Kaya et al., 2015, Liu et al., 2015, Millet et al., 2015), confers resistance to cellular stress (Chen et al., 2012, Duncan et al., 2012, Kaya et al., 2015), and arguably imparts invasiveness on embryos necessary for implantation and deep placentation. Recent reports demonstrated unequivocally that embryos harboring complex mosaic aneuploidies can give rise to healthy offspring, both in humans and, experimentally, in mice (Bolton et al., 2016, Greco et al., 2015).

Several mechanisms ensure survival of mosaic embryos, including self-correction through apoptosis and possibly sequestration of aneuploid cells into the trophoblast lineage (Bolton et al., 2016) (Figure 1). However, invasiveness combined with the exceptional diversity of human embryos necessitates the need for additional, external (i.e. uterine) selection to limit

the risk of maternal investment in a failing pregnancy (Gellersen and Brosens, 2014, Macklon and Brosens, 2014). The first evidence that the endometrium is an intrinsic biosensor of embryo quality actually originated from studies in cattle. Microarray studies showed that the pregnant bovine endometrium mounts a transcriptional response that is distinct for embryos generated by artificial insemination, IVF, or somatic cell nuclear transfer (Mansouri-Attia et al., 2009). Decidualizing human endometrial stromal cells have since emerged as exquisite sensors that respond to as yet unidentified embryonic serine proteases in a manner that either supports further development (positive selection) or ensures rapid disposal through menstruation-like shedding (negative selection) (Brosens et al., 2014b). Quality control may not necessarily cease once the conceptus is embedded in the endometrium but likely continues throughout the first trimester of pregnancy. For example, the gradual shift from ovarian to placental progesterone production arguably means that the endometrium will *de facto* select against embryos that are perceived to lack fitness because of insufficient human chorionic gonadotropin production. Similarly, the onset of placental perfusion around week 10 of pregnancy causes dramatic changes in local oxygen tension and triggers bursts of free radicals (Burton et al., 1999) (Figure 1), effectively stress-testing the feto-maternal interface. Thus, suboptimal selection at implantation inevitably increases the risk of clinical miscarriage; and conversely, once all selection pressures have been endured successfully by the end of the 1st trimester, the likelihood of further catastrophic failure drops markedly (Figure 1). The corollary of inefficient embryo selection at implantation is rapid conception, defined by short time-to pregnancy interval. It has been estimated that 40% of recurrent miscarriage patients are superfertile, defined by the fact that each pregnancy is achieved within 3 cycles (Orlando and Coulam, 2014, Teklenburg et al., 2010, Salker et al., 2010).

Endometrial stem cells are perhaps the least appreciated and least understood drivers of reproductive plasticity. Considering the unrivalled regenerative capacity of the endometrium, it is remarkable, if not baffling, that the first experimental study demonstrating the presence of resident endometrial mesenchymal stem cells (eMSCs) was reported only 12 years ago (Chan et al., 2004). eMSCs are abundant, multipotent, immuno-privileged, and highly regenerative in various pre-clinical models of disease (reviewed in Mutlu et al., 2015).

In this commentary, we explore the role of endometrial MSCs in effecting one of the most salient aspects of human reproduction, i.e. the ability to achieve a live birth after multiple pregnancy failures.

Persistent reproductive failure

One of the most disappointing aspects of modern reproductive medicine is the pervasive puerile view of implantation and early pregnancy. Patients suffering from reproductive failure, i.e. repeated IVF implantation failures or recurrent miscarriage, continue to be subjected to a battery of screening tests for subclinical 'disorders', which are presumed to converge somehow onto a 'fragile' implanting embryo, causing reproductive failure. Numerous anatomical, endocrine, immunological, thrombophilic and genetic perturbations have been invoked to explain persistent reproductive failure (Agenor and Bhattacharya, 2015, Rai and Regan, 2006). Yet every diagnostic test currently in clinical practice lacks specificity, meaning that many women with normal pregnancies also test positive. Nevertheless, it remains standard practice to label a 'positive' test as 'causal', ignoring the lack of clinical evidence, biological plausibility, or the absence of interventions that are even remotely effective. In the absence of a 'positive' test, many clinicians resort to exalting the virtues of vitamins, micronutrients and other soft interventions. Others advocate an interventional approach, using a range of combinatory empirical treatments (e.g. heparin, aspirin, steroids, human chorionic gonadotropin, intravenous immunoglobulin, hydroxychloroquine, intralipids, TNFα inhibitors, etc.) as well as pre-implantation genetic screening of IVF embryos. None of these interventions have been conclusively shown to improve reproductive success; and some may well be harmful.

And yet, despite this lamentable state of affairs, most women suffering either repeated implantation failure or recurrent miscarriage do achieve a successful pregnancy (Saravelos and Regan, 2014, Brigham et al., 1999, Ogasawara et al., 2000, Practice Committee of the American Society for Reproductive, 2012, Vlaanderen, 2014), irrespective of treatment. For example, several randomized control trials on recurrent miscarriage, defined here as 3 consecutive pregnancy losses, reported life-births rate of 65% or more in the placebo group (Coomarasamy et al., 2015, Pasquier et al., 2015). In recurrent miscarriage, the incidence of euploid fetal loss increases with each additional miscarriage, whereas the likelihood of a future successful pregnancy gradually decreases (Ogasawara et al., 2000). These observations indicate that RM is a graded disorder with the level of severity defined by the number of previous pregnancy losses. Nevertheless, even after 5 consecutive miscarriages, the

likelihood of a life-birth in the subsequent pregnancy remains in excess of 50% (Rai and Regan, 2006, Brigham et al., 1999).

A parsimonious explanation for these clinical observations is that embryo-endometrial interactions are intrinsically dynamic and capable of adapting from pregnancy to pregnancy to ensure reproductive success. The more severe the defect, however, the higher the likelihood of repeated pregnancy failures in consecutive conception cycles.

The decidualizing endometrium in recurrent miscarriage

Much of the work on the endometrium in the context of recurrent miscarriage has focused on decidualization, an obligatory transformative process for pregnancy in all mammalian species where implantation involves breaching of the luminal endometrial epithelium by the conceptus (Ramsey et al., 1976). The decidual process is foremost characterized by transformation of endometrial fibroblasts into specialized epithelioid cells. In parallel, the endometrium undergoes extensive remodeling, effected by the influx of specialized immune cells, predominantly uterine natural killer cells and macrophages (reviewed by Gellersen and Brosens, 2014). Associated vascular changes then prepare the human endometrium for endovascular trophoblast invasion and the formation of a functional haemochorial placenta (Brosens et al., 2002). Decidual cells encapsulate and safeguard the conceptus against various stressors. For example, stress-induced signaling through c-Jun N-terminal kinase and p38 mitogen-activated protein kinase pathways is selectively inactivated upon decidualization of human endometrial stromal cells (Leitao et al., 2010, Yoshino et al., 2003, Leitao et al., 2011). In parallel with a marked induction of various free radical scavengers, silencing of stress-signaling pathways renders decidual cells extraordinarily resistant to oxidative cell death (Kajihara et al., 2006). Moreover, circadian oscillations within the endometrium are firmly disabled upon decidualization (Muter et al., 2015), which further isolates the implanting blastocyst from the maternal environment. Decidual cells are also gatekeepers and chief modulators of local immune cells at the embryo-endometrial interface. In pregnancy, decidual cells also actively prevent influx of antigen-specific cytotoxic T lymphocytes by silencing of genes encoding key chemokines (Nancy et al., 2012).

Importantly, decidualization is not an all or nothing phenomenon. Instead, differentiating human endometrial stromal cells transit through distinct functional phenotypes upon

transformation into decidual cells (Lucas et al., 2016, Salker et al., 2012). This transitional pathway, which can be recapitulated in culture, is characterized initially by an acute pro-inflammatory response, lasting several days. Release by differentiating endometrial stromal cells of 'alarmins' like interleukin-33, a potent activator of the innate immune system, has emerged as an important driver of this transient inflammatory process (Salker et al., 2012). These inflammatory mediators in turn up-regulate the expression of key implantation genes, including leukemia inhibitory factor, interleukin 1-β, heparin binding EGF, bone morphogenetic protein 2, wingless-related MMTV integration site 4, and homeobox protein 10. Acquisition of a mature decidual phenotype curtails the inflammatory response through induction of anti-inflammatory soluble decoy receptors (Salker et al., 2012) and pronounced upregulation of 11β-hydroxysteroid dehydrogenase type 1, leading to increased local cortisol production (Kuroda et al., 2013a, Kuroda et al., 2013b). Decidual factors such as LEFTY2 contribute to the active closure of the window of receptivity (Tang et al., 2005, Tabibzadeh et al., 2000). As aforementioned, mature decidual cells are exquisitely responsive to embryo signals, especially trypsin-like proteases, and engage in active rejection by triggering menstruation-like shedding mediated by a decidual stress response (Brosens et al., 2014a). Taken together, these observations illustrate how the decidualization pathway contributes to the functional transition of the endometrium from a non-receptive (early-secretory phase) to a receptive (mid-secretory phase) and then a selective (late-secretory phase) state.

The hallmark of the endometrium in recurrent miscarriage is a disordered and prolonged pro-inflammatory decidual response. This excessive inflammatory response in turn prolongs the 'window of receptivity', promotes out-of-phase implantation, and disables embryo selection (Salker et al., 2010, Salker et al., 2011, Salker et al., 2012, Lucas et al., 2016). Consequently, poor quality embryos are not disposed of in a timely manner and high-quality embryos implant in an unsupportive environment. Both scenarios lead to clinical miscarriage.

'Memory' of endometrial stromal cells

A truly prodigious finding, reported first 10 years ago, is that human endometrial stromal cells from individual patients closely phenocopy the decidual response *in vivo* upon differentiation in culture (Klemmt et al., 2006). Aberrant decidualization in culture has not only been reported for recurrent miscarriage patients (Francis et al., 2006, Salker et al., 2010)

but also for endometriosis (Klemmt et al., 2006, Aghajanova et al., 2011, Ahn et al., 2016) and polycystic ovary syndrome (Piltonen et al., 2015, Piltonen et al., 2013). However, while progesterone resistance, defined by the refractoriness of cultured human endometrial stromal cells to deciduogenic cues (Barragan et al., 2016), characterizes endometriosis patients; an excessive and prolonged inflammatory decidual response is typically associated with recurrent miscarriage (Salker et al., 2012).

We hypothesized that an epigenetic mechanism may underlie this pathological 'memory' of endometrial stromal cells associated with recurrent miscarriage. Consequently, we sequenced immunoprecipitated methylated DNA (MeDIP-seq) to compare the global cytosine methylation profiles in primary cultures established from mid-luteal biopsies from recurrent miscarriage patients and control subjects (Lucas et al., 2016). Disappointingly, the methylation signature at CG dinucleotides, the most common context of DNA methylation, was largely similar between the clinical groups, although there were notable differences in genes associated with decidualization and implantation processes, including the progesterone receptor co-activator high-mobility group box 2 (HMGB2) (Boonyaratanakornkit et al., 1998).

Some MeDIP-seq analysis pipelines utilize protocols based on the assumption that methylation is confined to CpG dinucleotides. When we re-analyzed the sequencing data using an unrestricted approach, a striking signature became apparent in endometrial stromal cells isolated from recurrent miscarriage samples, characterized by hypomethylation of 2,741 loci. These differentially methylated regions (DMRs) overwhelmingly mapped to CA-rich regions that were largely devoid of CpG dinucleotides. Many DMRs not only clustered within 15 Mb of the telomeres but also contained several DNA motifs that were selectively hypomethylated in recurrent miscarriage patients (Lucas et al., 2016).

Methylation at CpH (H=A, C, T) is an epigenetic hallmark of stem cells, embryos and gametes (Ramsahoye et al., 2000, Shirane et al., 2013). It is lost in most somatic tissues but can be re-established upon pluripotency reprogramming of somatic cells (Ziller et al., 2011). Hence, we reasoned that the global CpH hypomethylation signature in endometrial stromal cells from recurrent miscarriage patients could be accounted for by lack of eMSCs or stemness.

234

235 **eMSCs in recurrent miscarriage**

236

237 The defining feature of the human sexual cycle, shared with few other mammals, is
238 menstruation. This remarkable phenomenon is triggered by falling progesterone levels in
239 species that exhibit cyclic decidualization of the endometrium independently of an implanting
240 embryo. MSCs regulate the main phases of wound healing via modulation of inflammatory
241 response, promotion of angiogenesis and stimulation of cell movement (reviewed by Wang et
242 al., 2016, Wong et al., 2015); thus a role for these cells in endometrial regeneration following
243 menstruation, miscarriage or parturition is obvious. Endometrial MSCs share the classic
244 properties of bone marrow MSCs, including clonogenicity, multipotency, the ability to
245 reconstitute endometrial stroma *in vivo*, and expression of surface markers that distinguish
246 them from leukocytes, hematopoietic and endothelial cells (reviewed by Gargett et al., 2016).

247

248 As described for other organs, eMSCs predominantly reside in the perivascular niche of both
249 the basal and functional layer (Ulrich et al., 2014, Masuda et al., 2012). Screening the
250 endometrium with a panel of perivascular markers identified SUSD2 (W5C5) as a powerful
251 marker for selection of clonogenic endometrial cells (Masuda et al., 2012). The
252 SUSD2/W5C5-positive cell population isolated by magnetic activated cell sorting constitutes
253 approximately 6-7% of endometrial stromal cells. The SUSD2/W5C5-positive cell fraction
254 contains on average 2-4% clonogenic cells (Masuda et al., 2012, Murakami et al., 2013).
255 However, clonogenic eMSCs can also be isolated from the SUSD2/W5C5-negative cell
256 fraction, although the relative abundance is much lower (0.7%) (Murakami et al., 2013). A
257 recent gene expression profiling study provided compelling evidence that clonogenic eMSCs
258 residing in the perivascular niche are the lineage precursors of the more committed non-
259 perivascular eMSCs (Barragan et al., 2016).

260

261 To explain the CpH hypomethylation signature in endometrial stromal cells of recurrent
262 miscarriage patients, we systematically measured the total number of freshly isolated
263 SUSD2/W5C5-positive cells, the abundance of clonogenic SUSD2/W5C5-positive eMSCs
264 and the abundance of clonogenic SUSD2/W5C5-negative eMSCs in mid-luteal biopsies
265 obtained from 31 recurrent miscarriage patients and 28 control subjects. The total number of
266 SUSD2/W5C5-positive cells did not differ between the study and control group. However,
267 recurrent miscarriage was associated with a 41% reduction in the abundance of clonogenic

SUSD2/W5C5-positive eMSCs, respectively. Strikingly, no clonogenic SUSD2/W5C5-negative eMSCs were recovered from 13 out of 31 (42%) recurrent miscarriage samples compared to 3 out of 28 (11%) control samples (Lucas et al., 2016).

Both the level of methylation and the abundance of clonogenic cells correlated inversely with the severity of the miscarriage phenotype, defined by the number of previous pregnancy losses. Furthermore, eMSC deficiency has profound ramifications downstream of the differentiation pathway by accumulating senescent cells in the endometrial stromal compartment. Cellular senescence is associated with distinct pro-inflammatory cytokines and chemokines, matrix metalloproteinases and growth factors, termed senescence-associated secretory phenotype (SASP) (Acosta et al., 2013). Importantly, induction of senescence in primary endometrial stromal cells triggered a blunted but prolonged inflammatory secretory response upon decidualization, akin to the secretory response of primary cultures from recurrent miscarriage patients and likely reflecting the contribution of SASP (Lucas et al., 2016).

Perspective

The advances in endometrial stem cell biology have generated innovative tools to accurately quantify and characterize eMSC populations associated with reproductive failure. Importantly, these advances are also re-writing our understanding of the biology of endometrial stromal cells. Rather than being a homogenous population, endometrial stromal cells, *in vivo* and *in vitro*, consist of a community of cells, ranging from quiescent and active MSCs, transit amplifying cells, mature fibroblasts and senescent cells. The observation that relative MSC deficiency and heightened cellular senescence is associated with recurrent miscarriage exemplifies that the decidual response, or more precisely the transitional decidual pathway, is determined by the balance of subpopulations that make up the community of stromal cells (Figure 2). By default, the constituents of this community, and thus the nature of the decidual response, changes with increasing distance away of the perivascular niche. A recent study illustrated this spatial organisation in the endometrium by demonstrating that perivascular SUSD2/W5C5-positive cells mount a distinct decidual response that could account for preferential homing of invading trophoblast to the spiral arteries (Murakami et al., 2014).

What causes eMSC deficiency in the uterus is a pertinent but as yet unanswered question. The properties and potential of adult stem cells are determined by a combination of intrinsic characteristics and a tissue-specific microenvironment. High Notch activity is a common niche feature and essential for cell-fate specification and maintenance of stem cells in a poised quiescent state (Cheung and Rando, 2013, Bjornson et al., 2012). For example, silencing of Notch signaling in skeletal cells leads to stem cell depletion and gives rise to muscles that lack the ability to regenerate in response to injury (Bjornson et al., 2012). By analogy, it seems plausible that pathological cues arising from dysmetabolic conditions associated with adverse pregnancy outcome, such as obesity, interfere with Notch signaling in the endometrium, thus gradually depleting the tissue of quiescent stem cells and rendering it vulnerable to damage in pregnancy. In support of this conjecture, a previous study reported that body mass index negatively correlates with cloning efficiency of endometrial SUSD2/W5C5-positive and -negative eMSCs (Murakami et al., 2013). Age, on the other hand, has no or little impact on the abundance of eMSCs (Murakami et al., 2013, Ulrich et al., 2014).

These observations lead to other pertinent questions. How does the endometrium maintain its MSC populations over 400 or so cycles of tissue breakdown and regeneration? And is expansion of the eMSC population required to accommodate pregnancy? A widely held assumption is that eMSCs residing in the basal layer, which is not shed during menstruation, maintain the regenerative capacity of the endometrium from menarche until the menopause and beyond. However, this seems unlikely as age does not seem to impact on the abundance of eMSCs. An alternative scenario is that influx of immune cells, and specifically uterine natural killer cells, during the luteal phase plays a key role in homeostatic balancing of the stromal subpopulations through selective clearance of senescent cells (Hoenicke and Zender, 2012). This attractive but as yet unproven scenario would link aberrant immune cell function to subsequent pregnancy failure without having to invoke a host-versus-graft response to the invading placental semi-allograft. Finally, it is not beyond the realms of possibility that decidual or trophoblast cues lead to expansion of eMSC population, in parallel with the expansion of the vascular bed. If this is the case, it would explain how the endometrium could self-correct over time and enable recurrent miscarriage patients to achieve a successful pregnancy after consecutive previous failures.

In summary, the discovery that eMSC deficiency is linked to recurrent miscarriage has provided new insights into the mechanisms of aberrant decidualization, lack of embryo selection and pregnancy failure. At the same time new questions have arisen regarding the mechanisms that control the maintenance of eMSCs from cycle to cycle and from pregnancy to pregnancy. Screening for eMSC deficiency may identify women at risk of recurrent reproductive failure and, conversely, harnessing the mechanisms that control endometrial stem cell populations may lead to effective interventions that reduce the physical and emotional trauma caused by recurrent miscarriage.

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Declaration of Interests

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

References

Acosta, JC, Banito, A, Wuestefeld, T, Georgilis, A, Janich, P, Morton, JP, Athineos, D, Kang, TW, Lasitschka, F, Andrulis, M, Pascual, G, Morris, KJ, Khan, S, Jin, H, Dharmalingam, G, Snijders, AP, Carroll, T, Capper, D, Pritchard, C, Inman, GJ, Longerich, T, Sansom, OJ, Benitah, SA, Zender, L & Gil, J 2013. A complex secretory program orchestrated by the inflammasome controls paracrine senescence. *Nat Cell Biol,* **15,** 978-90.

Agenor, A & Bhattacharya, S 2015. Infertility and miscarriage: common pathways in manifestation and management. *Womens Health (Lond Engl),* **11,** 527-41.

Aghajanova, L, Tatsumi, K, Horcajadas, JA, Zamah, AM, Esteban, FJ, Herndon, CN, Conti, M & Giudice, LC 2011. Unique transcriptome, pathways, and networks in the human endometrial fibroblast response to progesterone in endometriosis. *Biol Reprod,* **84,** 801-15.

Ahn, JI, Yoo, JY, Kim, TH, Kim, YI, Ferguson, SD, Fazleabas, AT, Young, SL, Lessey, BA, Ahn, JY, Lim, JM & Jeong, JW 2016. cAMP-Response Element-Binding 3-Like Protein 1 (CREB3L1) is Required for Decidualization and its Expression is Decreased in Women with Endometriosis. *Curr Mol Med,* **16,** 276-87.

Barragan, F, Irwin, JC, Balayan, S, Erikson, DW, Chen, JC, Houshdaran, S, Piltonen, TT, Spitzer, TL, George, A, Rabban, JT, Nezhat, C & Giudice, LC 2016. Human Endometrial Fibroblasts Derived from Mesenchymal Progenitors Inherit Progesterone Resistance and Acquire an Inflammatory Phenotype in the Endometrial Niche in Endometriosis. *Biol Reprod*.

Bjornson, CR, Cheung, TH, Liu, L, Tripathi, PV, Steeper, KM & Rando, TA 2012. Notch signaling is necessary to maintain quiescence in adult muscle stem cells. *Stem cells,* **30,** 232-42.

Bolton, H, Graham, SJ, Van Der Aa, N, Kumar, P, Theunis, K, Fernandez Gallardo, E, Voet, T & Zernicka-Goetz, M 2016. Mouse model of chromosome mosaicism reveals lineage-specific depletion of aneuploid cells and normal developmental potential. *Nature communications,* **7,** 11165.

Boonyaratanakornkit, V, Melvin, V, Prendergast, P, Altmann, M, Ronfani, L, Bianchi, ME, Taraseviciene, L, Nordeen, SK, Allegretto, EA & Edwards, DP 1998. High-mobility group chromatin proteins 1 and 2 functionally interact with steroid hormone receptors to enhance their DNA binding in vitro and transcriptional activity in mammalian cells. *Mol Cell Biol,* **18,** 4471-87.

Brigham, SA, Conlon, C & Farquharson, RG 1999. A longitudinal study of pregnancy outcome following idiopathic recurrent miscarriage. *Hum Reprod,* **14,** 2868-71.

Brosens, JJ, Pijnenborg, R & Brosens, IA 2002. The myometrial junctional zone spiral arteries in normal and abnormal pregnancies: a review of the literature. *Am J Obstet Gynecol,* **187,** 1416-23.

Brosens, JJ, Salker, MS, Teklenburg, G, Nautiyal, J, Salter, S, Lucas, ES, Steel, JH, Christian, M, Chan, YW, Boomsma, CM, Moore, JD, Hartshorne, GM, Sucurovic, S, Mulac-Jericevic, B, Heijnen, CJ, Quenby, S, Koerkamp, MJ,

Holstege, FC, Shmygol, A & Macklon, NS 2014a. Uterine selection of human embryos at implantation. *Sci Rep,* **4,** 3894.

Brosens, JJ, Salker, MS, Teklenburg, G, Nautiyal, J, Salter, S, Lucas, ES, Steel, JH, Christian, M, Chan, YW, Boomsma, CM, Moore, JD, Hartshorne, GM, Sucurovic, S, Mulac-Jericevic, B, Heijnen, CJ, Quenby, S, Koerkamp, MJ, Holstege, FC, Shmygol, A & Macklon, NS 2014b. Uterine selection of human embryos at implantation. *Scientific reports,* **4,** 3894.

Burton, GJ, Jauniaux, E & Watson, AL 1999. Maternal arterial connections to the placental intervillous space during the first trimester of human pregnancy: the Boyd collection revisited. *Am J Obstet Gynecol,* **181,** 718-24.

Chan, RW, Schwab, KE & Gargett, CE 2004. Clonogenicity of human endometrial epithelial and stromal cells. *Biol Reprod,* **70,** 1738-50.

Chen, G, Bradford, WD, Seidel, CW & Li, R 2012. Hsp90 stress potentiates rapid cellular adaptation through induction of aneuploidy. *Nature,* **482,** 246-50.

Cheung, TH & Rando, TA 2013. Molecular regulation of stem cell quiescence. *Nature reviews. Molecular cell biology,* **14,** 329-40.

Coomarasamy, A, Williams, H, Truchanowicz, E, Seed, PT, Small, R, Quenby, S, Gupta, P, Dawood, F, Koot, YE, Bender Atik, R, Bloemenkamp, KW, Brady, R, Briley, AL, Cavallaro, R, Cheong, YC, Chu, JJ, Eapen, A, Ewies, A, Hoek, A, Kaaijk, EM, Koks, CA, Li, TC, Maclean, M, Mol, BW, Moore, J, Ross, JA, Sharpe, L, Stewart, J, Vaithilingam, N, Farquharson, RG, Kilby, MD, Khalaf, Y, Goddijn, M, Regan, L & Rai, R 2015. A Randomized Trial of Progesterone in Women with Recurrent Miscarriages. *N Engl J Med,* **373,** 2141-8.

Duncan, AW, Hanlon Newell, AE, Bi, W, Finegold, MJ, Olson, SB, Beaudet, AL & Grompe, M 2012. Aneuploidy as a mechanism for stress-induced liver adaptation. *J Clin Invest,* **122,** 3307-15.

Fragouli, E, Alfarawati, S, Spath, K, Jaroudi, S, Sarasa, J, Enciso, M & Wells, D 2013. The origin and impact of embryonic aneuploidy. *Hum Genet*.

Francis, J, Rai, R, Sebire, NJ, El-Gaddal, S, Fernandes, MS, Jindal, P, Lokugamage, A, Regan, L & Brosens, JJ 2006. Impaired expression of endometrial differentiation markers and complement regulatory proteins in patients with recurrent pregnancy loss associated with antiphospholipid syndrome. *Mol Hum Reprod,* **12,** 435-42.

Gargett, CE, Schwab, KE & Deane, JA 2016. Endometrial stem/progenitor cells: the first 10 years. *Hum Reprod Update,* **22,** 137-63.

Gellersen, B & Brosens, JJ 2014. Cyclic decidualization of the human endometrium in reproductive health and failure. *Endocr Rev,* **35,** 851-905.

Greco, E, Minasi, MG & Fiorentino, F 2015. Healthy Babies after Intrauterine Transfer of Mosaic Aneuploid Blastocysts. *N Engl J Med,* **373,** 2089-90.

Haig, D 1993. Genetic conflicts in human pregnancy. *The Quarterly review of biology,* **68,** 495-532.

Hoenicke, L & Zender, L 2012. Immune surveillance of senescent cells--biological significance in cancer- and non-cancer pathologies. *Carcinogenesis,* **33,** 1123-6.

Kajihara, T, Jones, M, Fusi, L, Takano, M, Feroze-Zaidi, F, Pirianov, G, Mehmet, H, Ishihara, O, Higham, JM, Lam, EW & Brosens, JJ 2006. Differential expression of FOXO1 and FOXO3a confers resistance to oxidative cell death upon endometrial decidualization. *Mol Endocrinol,* **20,** 2444-55.

Kaya, A, Gerashchenko, MV, Seim, I, Labarre, J, Toledano, MB & Gladyshev, VN 2015. Adaptive aneuploidy protects against thiol peroxidase deficiency by

increasing respiration via key mitochondrial proteins. *Proc Natl Acad Sci U S A,* **112,** 10685-90.

Klemmt, PA, Carver, JG, Kennedy, SH, Koninckx, PR & Mardon, HJ 2006. Stromal cells from endometriotic lesions and endometrium from women with endometriosis have reduced decidualization capacity. *Fertil Steril,* **85,** 564-72.

Kuroda, K, Venkatakrishnan, R, James, S, Sucurovic, S, Mulac-Jericevic, B, Lucas, ES, Takeda, S, Shmygol, A, Brosens, JJ & Quenby, S 2013a. Elevated periimplantation uterine natural killer cell density in human endometrium is associated with impaired corticosteroid signaling in decidualizing stromal cells. *J Clin Endocrinol Metab,* **98,** 4429-37.

Kuroda, K, Venkatakrishnan, R, Salker, MS, Lucas, ES, Shaheen, F, Kuroda, M, Blanks, A, Christian, M, Quenby, S & Brosens, JJ 2013b. Induction of 11beta-HSD 1 and Activation of Distinct Mineralocorticoid Receptor- and Glucocorticoid Receptor-Dependent Gene Networks in Decidualizing Human Endometrial Stromal Cells. *Molecular endocrinology,* **27,** 192-202.

Leitao, B, Jones, MC, Fusi, L, Higham, J, Lee, Y, Takano, M, Goto, T, Christian, M, Lam, EW & Brosens, JJ 2010. Silencing of the JNK pathway maintains progesterone receptor activity in decidualizing human endometrial stromal cells exposed to oxidative stress signals. *FASEB J,* **24,** 1541-51.

Leitao, BB, Jones, MC & Brosens, JJ 2011. The SUMO E3-ligase PIAS1 couples reactive oxygen species-dependent JNK activation to oxidative cell death. *FASEB J*.

Liu, G, Yong, MY, Yurieva, M, Srinivasan, KG, Liu, J, Lim, JS, Poidinger, M, Wright, GD, Zolezzi, F, Choi, H, Pavelka, N & Rancati, G 2015. Gene Essentiality Is a Quantitative Property Linked to Cellular Evolvability. *Cell,* **163,** 1388-99.

Lucas, ES, Dyer, NP, Murakami, K, Hou Lee, Y, Chan, YW, Grimaldi, G, Muter, J, Brighton, PJ, Moore, JD, Patel, G, Chan, JK, Takeda, S, Lam, EW, Quenby, S, Ott, S & Brosens, JJ 2016. Loss of Endometrial Plasticity in Recurrent Pregnancy Loss. *Stem Cells,* **34,** 346-56.

Macklon, NS & Brosens, JJ 2014. The human endometrium as a sensor of embryo quality. *Biol Reprod,* **91,** 98.

Mansouri-Attia, N, Sandra, O, Aubert, J, Degrelle, S, Everts, RE, Giraud-Delville, C, Heyman, Y, Galio, L, Hue, I, Yang, X, Tian, XC, Lewin, HA & Renard, JP 2009. Endometrium as an early sensor of in vitro embryo manipulation technologies. *Proc Natl Acad Sci U S A,* **106,** 5687-92.

Masuda, H, Anwar, SS, Buhring, HJ, Rao, JR & Gargett, CE 2012. A novel marker of human endometrial mesenchymal stem-like cells. *Cell Transplant,* **21,** 2201-14.

Millet, C, Ausiannikava, D, Le Bihan, T, Granneman, S & Makovets, S 2015. Cell populations can use aneuploidy to survive telomerase insufficiency. *Nature communications,* **6,** 8664.

Murakami, K, Bhandari, H, Lucas, ES, Takeda, S, Gargett, CE, Quenby, S, Brosens, JJ & Tan, BK 2013. Deficiency in clonogenic endometrial mesenchymal stem cells in obese women with reproductive failure--a pilot study. *PLoS One,* **8,** e82582.

Murakami, K, Lee, YH, Lucas, ES, Chan, YW, Durairaj, RP, Takeda, S, Moore, JD, Tan, BK, Quenby, S, Chan, JK, Gargett, CE & Brosens, JJ 2014. Decidualization induces a secretome switch in perivascular niche cells of the human endometrium. *Endocrinology,* **155,** 4542-53.

Muter, J, Lucas, ES, Chan, YW, Brighton, PJ, Moore, JD, Lacey, L, Quenby, S, Lam, EW & Brosens, JJ 2015. The clock protein period 2 synchronizes mitotic expansion

and decidual transformation of human endometrial stromal cells. *FASEB J,* **29,** 1603-14.

Mutlu, L, Hufnagel, D & Taylor, HS 2015. The endometrium as a source of mesenchymal stem cells for regenerative medicine. *Biol Reprod,* **92,** 138.

Nancy, P, Tagliani, E, Tay, CS, Asp, P, Levy, DE & Erlebacher, A 2012. Chemokine gene silencing in decidual stromal cells limits T cell access to the maternal-fetal interface. *Science,* **336,** 1317-21.

Ogasawara, M, Aoki, K, Okada, S & Suzumori, K 2000. Embryonic karyotype of abortuses in relation to the number of previous miscarriages. *Fertil Steril,* **73,** 300-4.

Orlando, J & Coulam, C 2014. Is superfertility associated with recurrent pregnancy loss? *Am J Reprod Immunol,* **72,** 549-54.

Pasquier, E, De Saint Martin, L, Bohec, C, Chauleur, C, Bretelle, F, Marhic, G, Le Gal, G, Debarge, V, Lecomte, F, Denoual-Ziad, C, Lejeune-Saada, V, Douvier, S, Heisert, M & Mottier, D 2015. Enoxaparin for prevention of unexplained recurrent miscarriage: a multicenter randomized double-blind placebo-controlled trial. *Blood,* **125,** 2200-5.

Piltonen, TT, Chen, J, Erikson, DW, Spitzer, TL, Barragan, F, Rabban, JT, Huddleston, H, Irwin, JC & Giudice, LC 2013. Mesenchymal stem/progenitors and other endometrial cell types from women with polycystic ovary syndrome (PCOS) display inflammatory and oncogenic potential. *J Clin Endocrinol Metab,* **98,** 3765-75.

Piltonen, TT, Chen, JC, Khatun, M, Kangasniemi, M, Liakka, A, Spitzer, T, Tran, N, Huddleston, H, Irwin, JC & Giudice, LC 2015. Endometrial stromal fibroblasts from women with polycystic ovary syndrome have impaired progesterone-mediated decidualization, aberrant cytokine profiles and promote enhanced immune cell migration in vitro. *Hum Reprod,* **30,** 1203-15.

Practice Committee of the American Society for Reproductive, M 2012. Evaluation and treatment of recurrent pregnancy loss: a committee opinion. *Fertil Steril,* **98,** 1103-11.

Rai, R & Regan, L 2006. Recurrent miscarriage. *Lancet,* **368,** 601-11.

Ramsahoye, BH, Biniszkiewicz, D, Lyko, F, Clark, V, Bird, AP & Jaenisch, R 2000. Non-CpG methylation is prevalent in embryonic stem cells and may be mediated by DNA methyltransferase 3a. *Proc Natl Acad Sci U S A,* **97,** 5237-42.

Ramsey, EM, Houston, ML & Harris, JW 1976. Interactions of the trophoblast and maternal tissues in three closely related primate species. *Am J Obstet Gynecol,* **124,** 647-52.

Refuerzo, JS 2012. Impact of multiple births on late and moderate prematurity. *Semin Fetal Neonatal Med,* **17,** 143-5.

Salker, M, Teklenburg, G, Molokhia, M, Lavery, S, Trew, G, Aojanepong, T, Mardon, HJ, Lokugamage, AU, Rai, R, Landles, C, Roelen, BaJ, Quenby, S, Kuijk, EW, Kavelaars, A, Heijnen, CJ, Regan, L, Macklon, NS & Brosens, JJ 2010. Natural selection of human embryos: impaired decidualization of the endometrium disables embryo-maternal interactions and causes recurrent pregnant loss. *PLoS One,* **April 22**.

Salker, MS, Christian, M, Steel, JH, Nautiyal, J, Lavery, S, Trew, G, Webster, Z, Al-Sabbagh, M, Puchchakayala, G, Foller, M, Landles, C, Sharkey, AM, Quenby, S, Aplin, JD, Regan, L, Lang, F & Brosens, JJ 2011. Deregulation of the serum- and

glucocorticoid-inducible kinase SGK1 in the endometrium causes reproductive failure. *Nat Med,* **17,** 1509-13.

Salker, MS, Nautiyal, J, Steel, JH, Webster, Z, Sucurovic, S, Nicou, M, Singh, Y, Lucas, ES, Murakami, K, Chan, YW, James, S, Abdallah, Y, Christian, M, Croy, BA, Mulac-Jericevic, B, Quenby, S & Brosens, JJ 2012. Disordered IL-33/ST2 activation in decidualizing stromal cells prolongs uterine receptivity in women with recurrent pregnancy loss. *PLoS One,* **7,** e52252.

Saravelos, SH & Regan, L 2014. Unexplained recurrent pregnancy loss. *Obstet Gynecol Clin North Am,* **41,** 157-66.

Shirane, K, Toh, H, Kobayashi, H, Miura, F, Chiba, H, Ito, T, Kono, T & Sasaki, H 2013. Mouse oocyte methylomes at base resolution reveal genome-wide accumulation of non-CpG methylation and role of DNA methyltransferases. *PLoS Genet,* **9,** e1003439.

Tabibzadeh, S, Mason, JM, Shea, W, Cai, Y, Murray, MJ & Lessey, B 2000. Dysregulated expression of ebaf, a novel molecular defect in the endometria of patients with infertility. *J Clin Endocrinol Metab,* **85,** 2526-36.

Tang, M, Taylor, HS & Tabibzadeh, S 2005. In vivo gene transfer of lefty leads to implantation failure in mice. *Hum Reprod,* **20,** 1772-8.

Taylor, J 2016. Evolutionary biology takes a hard look at IVF and human conception. *The Evolution and Medicine Review*.

Taylor, TH, Gitlin, SA, Patrick, JL, Crain, JL, Wilson, JM & Griffin, DK 2014. The origin, mechanisms, incidence and clinical consequences of chromosomal mosaicism in humans. *Hum Reprod Update,* **20,** 571-81.

Teklenburg, G, Salker, M, Heijnen, C, Macklon, NS & Brosens, JJ 2010. The molecular basis of recurrent pregnancy loss: impaired natural embryo selection. *Mol Hum Reprod,* **16,** 886-95.

Ulrich, D, Tan, KS, Deane, J, Schwab, K, Cheong, A, Rosamilia, A & Gargett, CE 2014. Mesenchymal stem/stromal cells in post-menopausal endometrium. *Hum Reprod,* **29,** 1895-905.

Vanneste, E, Voet, T, Le Caignec, C, Ampe, M, Konings, P, Melotte, C, Debrock, S, Amyere, M, Vikkula, M, Schuit, F, Fryns, JP, Verbeke, G, D'hooghe, T, Moreau, Y & Vermeesch, JR 2009. Chromosome instability is common in human cleavage-stage embryos. *Nat Med,* **15,** 577-83.

Vlaanderen, W 2014. Recurrent miscarriage: guidelines could be improved. *Hum Reprod,* **29,** 1344-5.

Wang, H & Dey, SK 2006. Roadmap to embryo implantation: clues from mouse models. *Nat Rev Genet,* **7,** 185-99.

Wang, Q, Ding, G & Xu, X 2016. Immunomodulatory functions of mesenchymal stem cells and possible mechanisms. *Histol Histopathol*, 11750.

Wong, SP, Rowley, JE, Redpath, AN, Tilman, JD, Fellous, TG & Johnson, JR 2015. Pericytes, mesenchymal stem cells and their contributions to tissue repair. *Pharmacol Ther,* **151,** 107-20.

Yoshino, O, Osuga, Y, Hirota, Y, Koga, K, Hirata, T, Yano, T, Ayabe, T, Tsutsumi, O & Taketani, Y 2003. Endometrial stromal cells undergoing decidualization down-regulate their properties to produce proinflammatory cytokines in response to interleukin-1 beta via reduced p38 mitogen-activated protein kinase phosphorylation. *J Clin Endocrinol Metab,* **88,** 2236-41.

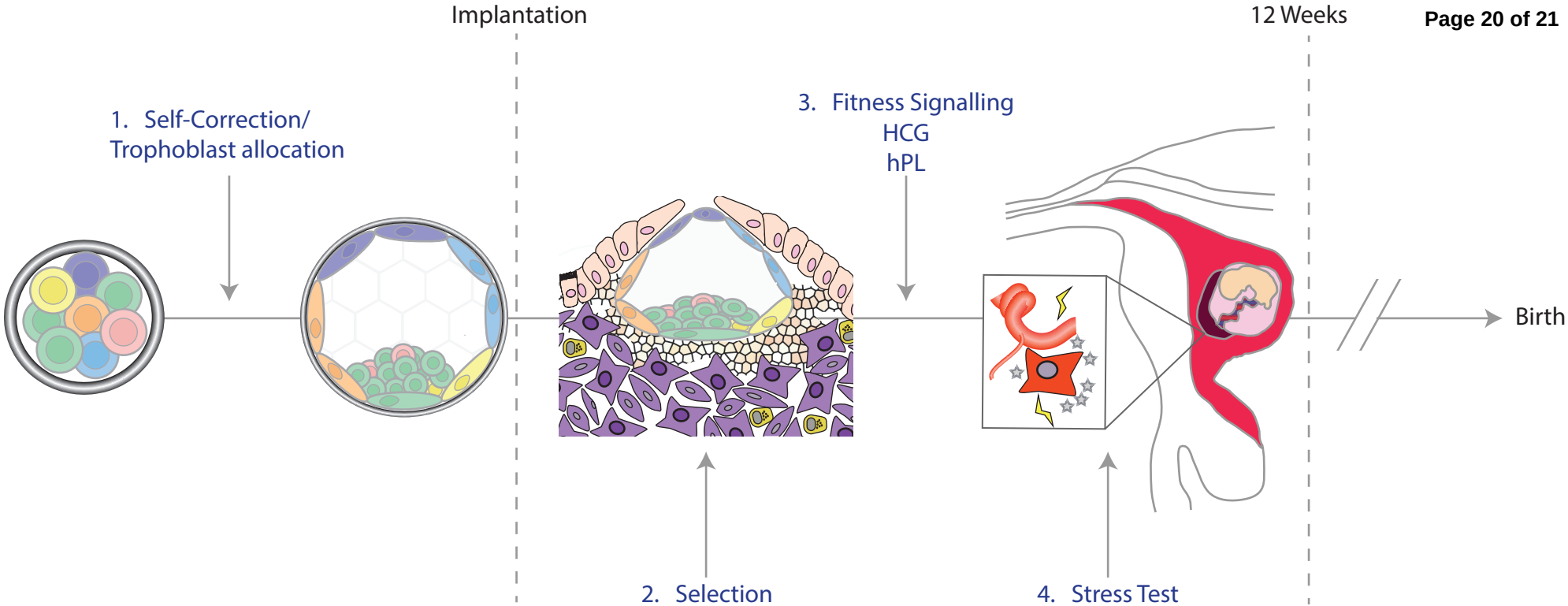
Ziller, MJ, Muller, F, Liao, J, Zhang, Y, Gu, H, Bock, C, Boyle, P, Epstein, CB, Bernstein, BE, Lengauer, T, Gnirke, A & Meissner, A 2011. Genomic

distribution and inter-sample variation of non-CpG methylation across human cell types. *PLoS Genet,* **7,** e1002389.

Figure legends

Figure 1. Embryo selection at implantation and recurrent miscarriage. Pre-implantation human embryos are remarkably diverse. Most human embryos are mosaic. Normal blastomeres are indicated in green whereas a different colour indicates a distinct aneuploidy. While embryonic mosaicism bestows adaptability onto the species through genetic diversity, it also increases the risk of prolonged maternal investment in a failing pregnancy. Several embryo-intrinsic and -extrinsic mechanisms operate in early pregnancy to limit this risk, including embryonic self-correction (1), biosensoring of embryo quality by decidualizing (purple) cells (2), corpus luteum rescue by placental fitness hormones such as hCG (3), and oxidative stress associated with the onset of placental perfusion at the end of the 1st trimester of pregnancy, effectively stress-testing the placental-decidual interface (4). Lack of embryo selection at implantation inevitably increases the risk of clinical miscarriage. Conversely, as a pregnancy transits to the 2nd trimester, selection pressure decreases and the risk of further miscarriage drops markedly.

Figure 2. Endometrial stromal compartment: a 'community' affair. The stromal compartment of the endometrium is a mixed community of cells of quiescent and active MSCs, transit-amplifying cells, mature fibroblasts and senescent cells. The composition of this community changes with increased distance from the perivascular niche. The effect of eMSC deficiency is amplified downstream of the perivascular niche. Failure of homeostatic balancing of the constituents of the stromal community accounts for aberrant decidualization associated with recurrent miscarriage.


Implantation
12 Weeks
1. Self-Correction/
Trophoblast allocation
3. Fitness Signalling
HCG
hPL
Birth
2. Selection
4. Stress Test

PERIVASCULAR

NON-PERIVASCULAR