



Contribution of immunology to build precision medicine in reproduction: present and future

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Abstract

Infertility affects millions of people of reproductive age. The failure of a blastocyst to implant is a leading cause of psychological distress. It became increasingly evident that an effective immune dialogue occurs at each step in the fluids surrounding the oocyte, the spermatozoa, the embryo, or the endometrium. Exploring and deciphering this dialogue could potentially help understand why 50% of healthy euploid blastocysts fail to implant. Introducing immunology into reproductive medicine requires a change of mindset to bring immune hypothesis to clinical applications. Implantation of an embryo requires a prepared uterus in order to dialogue with the embryo, which is able to express and repair itself. Exploring the uterine immune profile of patients with previous implantation failures (IF) or recurrent miscarriages (RM) has already been developed and is under evaluation as a precision tool to equilibrate the uterine environment before implantation to increase the subsequent live birth rate after the embryo transfer. Immunology may also be fundamental in the future to identify through non-invasive procedure the competence of oocytes or embryos through reliable immune biomarkers quantified in follicular fluids or embryo supernatants during the *in vitro* fertilization (IVF) process. Non-invasive biomarkers would allow physicians to identify competent oocytes or embryos based on their ability to communicate with the mother and their energetic potential for all the self-repair processes that should occur during the preimplantation and the implantation period. This area of research is only beginning.

Keywords

Infertility, immunology, embryo implantation, oocyte quality, reproduction

Introduction

It has been documented that millions of people of reproductive age are impacted by infertility worldwide. About 48 million couples in addition to 186 million people suffer from infertility around the globe impacting

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15% of couples having unprotected intercourse [1]. Inability to conceive is identified as a major root cause of stress, low self-esteem, depression in addition to aggressive social behavior [2, 3]. Studies suggest that the birth rate in the USA has been improved by 1% to 2% since 1978 due to the *in vitro* fertilization (IVF) resulting in more than 8 million newborns [European Society of Human Reproduction and Embryology (ESHRE, 2018)]. Although IVF has made good progress, the successful conception rate due to embryo transfer (ET) has stalled leaving about 50% of good quality blastocysts failing to implant [4].

Effective hemochorial placentation requires coordination between a healthy embryo and a prepared endometrium to help the embryo implant successfully. For this process to succeed, effective collaboration between the oocyte and the spermatozoa followed by the collaboration between the embryo and the endometrium must take place. Studies increasingly reveal that an effective interaction takes place at each step through extracellular vesicles (EVs) [5], microRNA (miRNA) [6], specific growth factors or cytokines present in the fluids surrounding the oocyte, the spermatozoa, the embryo or the endometrium. Analyzing and understanding this interaction could be the answer to understanding the reason why 50% of healthy blastocysts fail to implant. For many years, the main reason for the high rate of failure has been identified—most of the embryos were not euploid and were the root cause of subsequent implantation failures (IF). The discovery of the Preimplantation Genetic Test Aneuploidy (PGT-A), is perceived as the dominant solution. Fifteen years down the line, PGT-A is identified as a solution if patients can produce enough oocytes to generate enough embryos to be analyzed, even if some patients with aneuploid embryos still fail to conceive [7]. More solutions to enhance implantation rates are yet to be discovered and developed.

Despite the research in immunology dedicated to exploring the dialogue between molecules, organs and cells, this field is almost unknown in reproductive medicine since no current applications have been approved by bodies such as American Society for Reproductive Medicine (ASRM) and ESHRE that generate clinical practice guidelines, and agreement by private and public agencies funding the cost of exploration and treatment to pay. Immune exploration and immunotherapy are often considered “experimental” especially for immune treatment perceived as based on anecdotal/observational data (and sometimes only theories about mechanisms causing pathophysiology).

Introducing immunology into reproductive medicine requires a change of mindset. Implantation of an embryo requires a uterus able to dialogue with an embryo able to express and repair itself. In this review, we will focus exclusively on this specific immune dialogue. First, we will detail our current knowledge and its subsequent applications regarding this dialogue on the uterine side by detailing the uterine immune profiling (UtimPro). In the second part, we will focus on the current emerging hypothesis and the future directions deciphering the “words” of oocytes, spermatozoa and embryos in this crucial, vital dialogue. Immunology may be useful in the future to identify through non-invasive procedure in individual follicular fluids (FFs) or embryo supernatants the competence to implant the corresponding oocyte or embryos. Non-invasive biomarkers would allow physicians to identify competent oocytes or embryos based on their ability to communicate with the mother and their energetic potential for all the self-repair processes that should occur during the preimplantation and implantation period.

Since embryo implantation is the key event of assisted reproduction, understanding the local immune events and interplay between the endometrium and the embryo that determine the success of implantation is crucial.

Immune profiling for a better understanding of the immune status on the endometrial side

The maternal immune system recognizes the foetus as a foreign entity; however, the foetus is not rejected [8]. Immunological tolerance should be present during pregnancy to protect against an aggressive maternal alloimmune response, according to Medawar [9], who hypothesized this fifty years ago before the discovery of T-cells. Maternal regulatory T (Treg) cells are known for their function in suppressing autoimmune responses [10] and their role in maintaining the pregnancy [11]. Therefore, their absence resulted in the foetus’ immunological rejection, causing the pregnancy to fail [10]. A study led by Zenclussen et al. [12] in 2005,

suggested that Treg cells are requisite for the tolerance of the allogeneic foetus by the mother. Along with the Treg cells, dendritic cells (DCs) play a vital role in implantation [13] which is linked to proper decidualization through coordinating the synchronization of uterine receptivity and embryo development [14]. Another study focused on the role of macrophages during implantation pointed out that luteal function is decreased when M1 and M2 macrophages (M1 and M2 are two subtypes of macrophages) are eliminated during implantation, causing IF [15]. Another study using a mouse model, led by Ono et al. [16] showed that it is the depletion of M2 macrophages that is related to IF as luteal function remains normal.

In this event, a crucial immune endometrial switch should occur during the implantation window not only to avoid the rejection of the semi-allogenic embryo but to promote its growth and nutrition [17].

The window of implantation occurs 7 days to 11 days after ovulation. Wilcox et al. [18] have previously reported that in most successful human pregnancies, the conceptus implants 8 to 10 days after ovulation. The risk of early pregnancy loss increases with later implantation. During the implantation window which occurs during the mid-luteal phase, important immune cells leave the uterus (such as B lymphocytes and some CD8⁺ lymphocytes) while others enter the endometrium. So at the time of “uterine receptivity”, almost all the immune cells belonging to the adaptive immune system escape from the endometrium while innate immune cells macrophage, uterine natural killer (uNK) cells, and DCs invade the endometrium [19]. The newly created immune environment plays a key role in embryo implantation [19]. uNK cells differ from circulating natural killer (NK) cells by their phenotype, their repertoire of activating and inhibiting receptors, the cytokines they secrete and their low cytotoxic potential [20]. Treg cells make the link between adaptive and local immune expression [10, 21]. Underactive immune cells fail to create the necessary implantation reaction; conversely, overactive immune cells can lead to the destruction of the endometrium and eventually the rejection of the embryo. This unique immune reaction (switch from an adaptive to an innate immune environment, specificity of the immune innate cells at the time of implantation) is essential for promoting embryo adhesion and its disruption is likely to obstruct implantation. This immune reaction is unique since it is highly specific to the uterine environment.

Early on, the ideal environment during the implantation window was thought to contain mainly T helper 2 (Th2, compared with Th1) cytokines, which would selectively allow the development of local mechanisms that promote immunotrophism and angiogenesis at the same time that they down-regulate inflammation and cytotoxic pathways [22]. Over time, the concept of pregnancy as a Th2 phenomenon has evolved. Both the absence and a large excess of Th1 cytokines are thought to be deleterious for implantation and placentation, as is the absence of Th2 cytokines [23, 24]. This transient immune switch, together with adequate uNK cell activation, appears fundamental in establishing local maternal tolerance and survival of the fetus. Interleukin (IL)-15 is directly involved in the post-ovulatory recruitment and maturation of uNK cells in the uterus [25] and it is essential for adequate Th2 cytokine production. Its effects on blood NK cells are different than on uNK cells. It does not convert them into potent cytolytic cells but instead participates directly in their maturation [26]. IL-18 is a Th2-promoting cytokine that, through the action of angiopoietin-2, allows the remodeling of the spiral arteries which is crucial for successful implantation [27, 28]. In human endometrium, IL-18 expression increases during the implantation window. Its main role is to remodel the maternal-side vasculature. However, IL-15 and IL-18, produced by either epithelial or stromal endometrial cells, are both bivalent: at high levels, and when not immuno-regulated, they behave as pro-inflammatory Th1 cytokines. The expression of IL-15 and IL-18, reflects the local production of interferon (IFN)- γ and tumor necrosis factor (TNF)- α , which can activate uNK cells to become cytotoxic [29]. We also focus on the role of TNF weak inducer of apoptosis (TWEAK) and its receptor, fibroblast growth factor-inducible molecule 14 (Fn-14), as immunoregulators of the local immune equilibrium. Using an animal model, we observed that TWEAK offers protection against the deleterious effects of a Th1 dominant (TNF- α -rich) environment during implantation and thus increases embryo survival [30]. TWEAK and its ligand, Fn-14, act as immune regulators of the Th1/Th2 cytokine balance in the human endometrium [31]. The UtimPro is an innovative tool that relies on the analysis of the local immune reaction occurring within the endometrium during the implantation window based on the quantitative evaluation by quantitative reverse transcription polymerase chain reaction (RT-qPCR) of IL-18, IL-15, TWEAK, Fn-14 and CD56.

UtimPro as a tool of precise medicine

TRT-qPCR was used to explore the uterine immune profile screening for women with infertility issues [repeated IF (RIF), recurrent miscarriages (RM)] using endometrial samples collected during the implantation window. The uterine immune profile is based on the quantitative analysis of five biomarkers (IL-18, IL-15, TWEAK, Fn-14, and CD56) that allow us to identify an immune profile for each patient.

This method of immunological endometrial profiling was patented as a technique to increase implantation success in assisted fertilization (PCT/EP2013/065355). In the patent, we previously defined the expression norms for our biomarkers in a fertile cohort and demonstrated that an immune profile is reproducible from one cycle to another over a period of six months if no surgery or pregnancy has occurred in the interim.

Endometrial immune profiles can be classified into four types:

—A balanced endometrial immune activation, as measured by IL-18/TWEAK and IL-15/Fn-14 messenger RNA (mRNA) ratios and a CD56⁺ cell count in the same range as previously defined in the fertile cohort.

We postulate in this profile that the endometrium is ready to go through the following steps of implantation: apposition, adhesion and invasion.

—A low endometrial immune activation profile is defined by low mRNA ratios for IL-15/Fn-14 (reflecting immature uNK cells) and/or IL-18/TWEAK, as well as the absence of uNK recruitment.

We postulate in this profile that the endometrium will not be fully effective for adhesion and promoting adequate immunotrophism during initial placentation.

—Over endometrial immune activation is defined by high mRNA ratios of IL-18/TWEAK and/or IL-15/Fn-14, as well as a high CD56⁺ cell count.

—A mixed endometrial immune profile is distinguished by a high mRNA ratio of IL-18/TWEAK (excess Th-1 cytokines) and a low IL-15/Fn-14 ratio (reflecting immature NK).

We postulate in these two later profiles, that the endometrium was not prepared for the crucial step of trophoblast invasion.

Hence, UtimPro is dedicated to answering if the endometrium is ready for an effective adhesion and a controlled invasion when the embryo will be transferred. When an immune profile is deregulated, recommendations are given to regulate the immunological profile for the patient.

Two populations seem to benefit from a uterine immune profile to increase their chance to be pregnant: patients with a past of embryo IF and patients with a history of RM [32–34].

We indeed observed endometrial immune dysregulation in 83.5% of 1,738 infertile patients in a large cohort study (over-immune local activation in 45%, low-immune local activation in 28%, mixed profile in 10.5%) [33]. A tailored treatment was recommended based on the immunological profile, either to inhibit uNK cell activation or to boost local mechanisms of embryo adhesion. Patients with a history of RIF or RM had significantly higher pregnancy rates after well-diagnosed deregulation and individualized therapy than non-deregulated patients (37.7% and 56%, respectively, against 26.9% and 24%, $P < 0.001$) [32].

If the immune profile reveals deregulation, recommendations are made to normalize the immune profile before the next IVF attempt. The biopsy for this test should be performed during the implantation window (mid-luteal phase), as a local immune switch occurs during this phase as explained before. We always recommended a cycle test to assess the efficacy of immunotherapy in patients who were deregulated with either an over-immune activation or a mixed profile. As a result, we advocated for the implementation of a new principle in order to confirm the disappearance of the observed deregulation under therapy prior to any transfer. The physician received the type of immune profile analyzed three weeks after the endometrial collection based on the immune uterine profile: no deregulation, low-immune activation, over-immune activation, mixed profile (immaturity of uNK with a Th-1 dominant environment) and some personalized suggestions to counteract the local disequilibrium if diagnosed based on the immune uterine profile (Table 1).

Table 1. Summary of suggested therapies according to the endometrial immune profile

Immune endometrial profile	GC	Intralipids	Luteal hCG	LMWH	Scratching	Supplementation in progesterone	Exposure to seminal plasma after ET
No dysregulation	-	-	-	-	-	-	-
Low immune activation	-	-	×	-	×	-	×
Over immune activation	×	×	-	×	-	×	-
Mixed profile	×	×	×	×	?	×	-

GC: glucocorticoids; LMWH: low molecular weight heparin; hCG: human chorionic gonadotropin; -: not recommended; ×: recommended; ?: depending on the case

Understanding immunotherapy for an effective precise medicine in IVF

Regarding the therapy and the personalized medicine, one size does not fit all. All Cochrane and metanalysis regarding immunotherapy (GC, LMWH and others) or mechanical procedure as scratching in IVF are considered not fully effective [35–37] since these were not based on a precise molecular diagnosis but only on a specific context of infertility. Fundamentalists scientists, on their part, highlight those efficacies of most, if not all, immunological therapies are unproven [38] and often prescribed on the false basis of a necessity of local/down-regulation of NK activity. Uterine and decidual NK cells are viewed solely as a danger for the conceptus and thus mostly, if not always, as killer cells [39, 40] and their central role of “good nanny” is neglected [41].

On our hand, each prescription needs to be motivated by deregulation observed in the endometrial immune profile of the patient. A drug is only shown to be active when a deregulated profile becomes regulated with the administered therapy.

GC are recommended as first-line treatment for women with an over endometrial immune activation or a mixed immune profile. The reason for prescribing GC for such immune profiles is that it has been reported in the past that these GC:

- Decrease the levels of Th-1 cytokines, NK cytotoxicity and hyperactivation of lymphokine-activated killer cells [42]. Limit the consequences of IL-15 mRNA overexpression [43].
- Modulate the Th1/Th2 balance when dominated by Th1 cytokines [44].

In case of resistance to corticosteroids, LMWH was an option because of its well-documented anticomplementary effect [45, 46]. In the year 2018, the endometrial immune profile of 55 patients with RIF, initially classified in over-immune activation, were tested under GC to evaluate the rate of normalization of the initial profile under GC [47].

Under GC, all immune biomarkers were normalized in 54.5% (30/55) of the RIF population with proven over-immune activation. On the contrary, we found a counterintuitive negative increase of immune biomarkers in 29.1% (16/55) of the cases and a partial normalization in 16.5% (9/55). On our hand, corticoids are active through a considerable rise in TWEAK; the IL-18/TWEAK mRNA ratio representing the Th-1/Th-2 local equilibrium was considerably lowered (0.29 *versus* 0.10, $P = 0.004$) in patients who normalized their ratio and were subsequently pregnant when given GC [47].

In an RIF scenario, testing the sensitivity to GC could be quite valuable. Fewer than half of RIF patients with immunological deregulation are likely to be GC responders who would benefit from GC treatment.

Intralipid therapy (ILT) is proposed as a second-line treatment for women with immune over-activation profiles who are unable to conceive with GC or whose immune profile remains deregulated despite GC [48]. While the precise mechanism by which immunomodulation is achieved remains unclear, some authors have suggested that ILT is able to inhibit pro-inflammatory mediators, particularly Th1 cells and has immunosuppressive properties on NK cells [49, 50].

One hundred and eight patients with a history of unexplained RIF or RM underwent endometrial profiling before and under ILT prior to their next ET. The objective of the slow perfusion of intralipid was to control the observed endometrial over-immune activation [48]. In patients successfully pregnant on Intralipid® who underwent sensitivity testing prior to ET, we documented a significant decrease in all three biomarkers used

to diagnose overactivation (CD56 cell count; IL-18/TWEAK, IL-14/Fn-14). Among the patients tested, 27% of the patients were resistant to intralipid.

In patients' responders to ILT, despite their history of RIF and/or RM, the delivery rate after ILT was excellent, reaching 55% (60/108). We suggest that Intralipid appears to be effective as a therapy in RIF patients who have hyper-immune activation of uNK cells and a normalization of the immune profile under therapy.

Scratching

Scratching illustrates the need to understand the immunological rationale that will guide our customization efforts since it is directly related to the immune reaction occurring during the implantation process. Biological rationale behind this procedure of local injury is linked to the fact that scratching enhances the expression of adhesion molecules at the next mid-luteal phase after scratching and enhances the adhesion step during embryo implantation. Such profiles of low local activation are only observed among 33% of the patients in implantation (384/1,145). When performed specifically in patients with a documented low activation, we were able to report an ongoing pregnancy rate of 38.5% at the subsequent ET (181/384). It became thus consistent that a blind usage of scratching especially at a time not triggering the immune reaction (as the follicular phase or just before the ET) would be negative [51, 52] or even deleterious and potentially harmful [53].

As early as 2010, Gnainsky et al. [54] reported that a local injury performed in the mid-luteal phase modified the endometrial expression occurring at the subsequent luteal phase regarding the recruitment of macrophage and DCs as well as the expression of adhesion molecules. Liang et al. [55] also reported the effect of a scratching performed in the mid-luteal phase promotes the local production of vascular endothelial growth factor (VEGF) during the next mid-luteal phase.

On our hand, in case of a local low activation profile with a low IL-15/Fn-14 mRNA ratio, which is regarded as uNK cell immaturity, endometrial scratching is indicated to trigger the local expression of adhesion molecules and IL-15 to promote uNK cells maturity and local angiogenesis. Previous research demonstrated that hCG triggers both the proliferation and the maturation of uNK cells and promotes local angiogenesis [56, 57].

Immunology and biomarkers of the future

A constructive and fruitful dialogue between the embryo and the uterus is a prerequisite. On the uterine side, UtimPro suggests that an equilibrated expression of our reproductive innate immunity is crucial to promoting the implantation. We must therefore make progress in understanding how the oocyte is expressed locally and then after fertilization we need to decipher the embryo expression.

FFs and cumulus cells

Oocyte quality strongly decreases with maternal age and is variable among patients. With the development of social vitrification, it becomes of prime importance to find reliable biomarkers of oocytes competence and potentiality. Oocyte morphology is unfortunately poorly informative [58]. Moreover, the maternal age of the first child does not stop increasing in our developed society. Aging is associated with increased production of reactive oxygen species (ROS), and other toxins which are likely to affect cellular and mitochondrial genome leading to imbalanced redox activity and aneuploidy [59]. Indirect studies focusing either on cumulus cells surrounding the oocyte or its individual FF that could be relevant fluids to explore some related parameters.

Cumulus cells are implicated in oocyte competence and development [60]. They interact directly with the oocyte to facilitate its maturation and are bathed in the same FF. Some studies identified differences regarding few markers of oxidative stress [61] or gene expression between euploid and aneuploid oocytes [62]. FF is also collected during oocyte retrieval. However, to avoid multiple vaginal punctures, FF is often pooled and not individually collected. If this technical problem was solved, individual concentration of some cytokines could be a relevant biomarker of oocyte competence.

Cytokines in FFs

FF seems to be a source of non-invasive oocyte quality indicators. A competent oocyte can be fertilized so it can promote the earliest stages of embryo development [63]. Only 30% of retrieved oocytes develop into a good quality embryo, and only 5% result in a live birth [64, 65]. Since morphology is not a good predictor of oocyte competence, evaluating oocyte quality remains a difficulty in IVF.

In IVF cycles, follicular granulocyte colony-stimulating factor (G-CSF) could be considered as a novel indicator of oocyte quality and embryo implantation. However, its function in reproduction is still poorly known. The significant increase in G-CSF during ovulation corresponds with the accumulation of follicular granulocytes, which stimulate G-CSF production by granulosa cells via paracrine interactions [66]. High follicular G-CSF concentrations may occur in follicles with good granulosa-leukocyte interactions, which could explain why embryos derived from these follicles have a higher implantation rate. G-CSF is a versatile cytokine best recognized for promoting neutrophil activation and differentiation during hematopoiesis [67–69]. It also plays an important role in immunity and inflammatory responses. In recent research using an ultra-sensitive enzyme-linked immunosorbent assay (ELISA), particularly designed for the detection of follicular G-CSF, the chance of successful implantation was found to be 3.3 times greater for embryos produced from high-G-CSF follicles than for those derived from low-G-CSF follicles [66]. It's still unclear how follicular G-CSF contributes to both oocyte maturation and ovulation, and how it's produced and secreted locally are two key questions. Serum G-CSF concentrations are highest after spontaneous ovulation [70] or during ovarian stimulation with gonadotrophins [71, 72].

Using a microbead-based multiplex sandwich immunoassay (Luminex Technology), we previously measured simultaneously cytokines and chemokines in each FF collected from individual follicles of oocytes subsequently fertilized and transferred after conventional ovarian hyperstimulation [29, 30]. The originality of the approach was to collect individual FFs and not pooled FFs and to ensure traceability of each sample until birth or failure of the attempt. This study revealed that the level of G-CSF in individual FF samples correlates with the implantation potential of the corresponding embryo. FF G-CSF was found to be the best predictor of subsequent birth [area under the receiver operating characteristic (AUCROC) = 0.81, $P < 0.0001$] when using a multivariate logistic regression model (including known covariates such as age, number of IVF attempts, antral follicle count and embryo quality). FF G-CSF was highly correlated with cytokines IL-7 and IL-17, suggesting key interactions within the follicle involving immune cells such as DCs and Treg cells. FF G-CSF may promote local maternal-fetal tolerance [73] or influence the oocyte's own mRNA levels or its potential for self-repair [74]. It might also interact with cells in the local microenvironment to induce cytokines and growth factors which are necessary for embryo development and implantation. Recently, high follicular G-CSF concentrations were correlated with the presence within the follicle of granulocytes emphasizing the importance of early granulosa-leukocyte interactions, which could explain the increased implantation rate of embryos arising from these follicles [66]. The clinical performance of an ultra-sensitive FF G-CSF immunoassay has been conducted to confirm the correlation between FF G-CSF concentration and live birth potential of the corresponding embryo after IVF. Authors suggest a 43% greater probability of implantation for optimal embryos with high G-CSF compared to the general implantation rate among optimal embryos [53].

miRNAs

miRNAs are small RNA molecules that circulate in biological fluids, such as in serum, plasma and other body fluids [75]; however, they have not been studied in terms of their contribution to female infertility or IVF. A study by Scalici et al. [76] suggests that these miRNAs may serve as new biomarkers for IVF patients, allowing for more individualized treatment. This study found that the expression of circulating miRNAs differed depending on the women's ovarian reserve status, gonadotropin treatments, and/or the outcome of IVF [76]. During the preimplantation stage, the embryo secretes miRNAs into the extracellular environment [77]. The implantation process is aided by miRNAs found in the endometrium and blastocysts [78].

Exosomes

EVs are a diverse family of lipid bilayer-derived nanovesicles produced by nearly all living cells [79]. Exosomes are nanoparticles (100 nm in diameter) released by cells that are capable of transporting small RNAs and mRNA to cells at a distance via the extracellular environment. We hypothesized that exosomes or slightly larger micro vesicles (100–300 nm) are released into the uterine cavity by the endometrial epithelium and that these vesicles contain specific miRNA that could be transferred to either the trophoctodermal cells of the blastocyst or the endometrial epithelial cells, thereby promoting implantation [80]. Exosomes are present in all the fluids (FF, endometrial fluid, seminal plasma, embryo supernatant) and are now considered as a major actor of this complex dialogue. From an immune point of view, they are described as nano-mediator of the immune response [79]. Exosomes from immunocytes may be crucial to prepare the maternal system to accept or reject the embryo. In function of their environment and maturity, immunocytes exosomes can stimulate or attenuate immune responses, accepting or rejecting the embryo.

Exosomes derived from DCs pre-treated with anti-inflammatory cytokines, such as IL-10 can suppress T cell activation and induce immune tolerance. Exosomes from DC's, B, or T cells can induce immunosuppression by various other mechanisms, including Fas-mediated apoptosis and downregulation of major histocompatibility complex (MHC)-peptide complexes. All these immune reactions would be essential for effective implantation.

The characterization and evaluation of the exosomes' composition and quantity might be considered as an important tool to identify the potential of the oocytes or the embryos. Moreover, this new area also opens some new perspectives of treatment of poor oocyte quality.

A study by Gurung et al. [81] reported that EVs are considered to be important mediators of implantation. However, a clear understanding of their role in mediating implantation has yet to be demonstrated or established. This study demonstrated, both *in vitro* (using human trophoctoderm stem cells) and *in vivo* (using a mouse model), that exosomes derived from human endometrial epithelial cells act on and alter the trophoctoderm of the blastocyst, thereby increasing the likelihood of successful implantation. They demonstrated that treating human trophoctoderm spheroids with hormonally primed endometrial cancer cell line 1 (ECC1)-derived exosomes increased spheroid adhesion to and outgrowth on endometrial epithelial cells, and findings in mouse embryos corroborated findings in humans [81]. Furthermore, exosomes aided in the development, hatching, and implantation of mouse embryos in the womb of the mother.

The biological roles of EVs in events occurring during the onset of pregnancy, as well as their involvement in the communication between the embryo and the maternal organism are demonstrated by Bridi et al. [82], in various mammalian species. EVs are important carriers of bioactive molecules that have the potential to influence key reproductive events during the early stages of pregnancy. EVs secreted by the oviduct and endometrium, as well as embryos, must be studied in greater depth to determine whether they can enter the peripheral circulation and modulate different pathways in maternal organisms [82]. This progress in our understanding related to this type of communication can, in turn, help physicians to develop more accurate methods for detecting pregnancies, abnormal pregnancies, and pre-pregnancy loss, as well as new technologies for modulating early embryo–maternal interactions.

Exosomes appear to be involved in a wide range of biological processes, depending on the cell from which they are secreted and the conditions under which they are released [80]. According to current evidence, exosomes fuse with the plasma membrane of the recipient cell and then release their contents into the target cell. According to some theories, binding occurs at the cell surface through specific receptors [30, 83], and internalization occurs through exocytosis. Given that exosomes have been shown to modulate the behavior of immune and cancer cells, both of which have actions that are similar to those of embryo implantation, elucidation of the steroidal regulation and the function of the exosomes in the uterine cavity will extend our understanding of the early embryo-maternal dialogue, with potential implications for our understanding of infertility and success rates of IVF procedures [80].

Conclusions

To conclude, reproductive immunology appears as the discipline of the future in reproduction since it is able to solve or at least give new insight regarding all problems still ongoing (embryo implantation and oocyte quality) in reproduction.

Abbreviations

DCs: dendritic cells

ET: embryo transfer

EVs: extracellular vesicles

FFs: follicular fluids

Fn-14: fibroblast growth factor-inducible molecule 14

GC: glucocorticoids

G-CSF: granulocyte colony-stimulating factor

hCG: human chorionic gonadotropin

IF: implantation failures

IL: interleukin

ILT: intralipid therapy

IVF: *in vitro* fertilization

LMWH: low molecular weight heparin

miRNA: microRNA

mRNA: messenger RNA

NK: natural killer

RIF: repeated implantation failures

RM: recurrent miscarriages

Th2: T helper 2

TNF: tumor necrosis factor

Treg: regulatory T

TWEAK: tumor necrosis factor weak inducer of apoptosis

uNK: uterine natural killer

UtimPro: uterine immune profiling

Declarations

Acknowledgments

We have written this article in memory of Gérard Chaouat, our mentor who passed away in 2021. He was one of those rare and pure men that it is an honor to know. As for all of us, his name evokes atypicality mixed with a dazzling intelligence in an eternally utopian spirit, generous and respectful of others. In our discipline of reproductive medicine, he fought against all odds to have the importance of innate immunity in reproduction accepted. He proved through his research the importance of the primordial immunological dialogue between the uterus and the embryo in the construction of our humanity (the placenta). His researches were decisive for the understanding of all obstetrical diseases and implantation disorders. Even today, if there is progress in our discipline, it will be by admitting, encompassing and overhanging the complexity of reproduction in order to go further.

Author contributions

AK and NL wrote this manuscript. NL interpreted the uterine immune profiles to personalize subsequent recommendations. AK performed the molecular analysis. MP and MR corrected the manuscript. All authors contributed to manuscript revision, read and approved the submitted version.

Conflicts of interest

NL and MP are both inventors (PCT/EP2013/065355) and created the start-up MatriceLab Innove. The authors declare no other financial or commercial conflicts of interest.

Ethical approval

Not applicable.

Consent to participate

Not applicable.

Consent to publication

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