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Uterine immune profiling for increasing live birth rate: A one-to-one matched cohort study

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ABSTRACT

Background: Embryo implantation remains the main limiting factor in IVF/ICSI program. Endometrial immune remodeling events begin before implantation and are a vital process for pregnancy, preparing future maternal immune tolerance and regulating the placentation process.
Methods: Between 2012 and 2014, 193 patients (analyzed group) enrolled in our IVF program benefitted of an endometrial immune profiling to determine if their uterus was immunologically ready to accept an embryo and, if not, the specific immune mechanisms involved. Subsequently, they had an effective embryo transfer (ET) with personalization of their treatments if an immune deregulation has been diagnosed. Each analyzed patient was paired to the closest patient included in the IVF program according to biological criteria (age, number of mature oocytes, stage and number of transferred embryo), which had no endometrial immune profiling (193 patients, non-analyzed group).
Finding: 78% of analyzed patients had a uterine immune dysregulation and therefore care personalization. Their corresponding live birth rate (LBR) was twice higher than observed in the matched control group with conventional cares (30.5% versus 16.6%, OR: 2.2 [1.27–3.83] p = 0.004) with a simultaneous drastic reduction of miscarriages per initiated pregnancy (17.9% versus 43.2%, OR: 0.29 [0.12–0.71], p = 0.005). 22% of analyzed patients had no dysregulation. They did not differ from their matched controls for LBR and miscarriages.
Conclusion: Uterine immune profiling enables an integrated approach of infertility that includes endometrial immunity as a key factor in planning personalized IVF/ICSI treatments. Personalization of treatment according to the woman's uterine immune balance produced a very significantly higher LBR.

1. Introduction

Of an estimated 11 million infertile Europeans (prevalence: 9%): half seek medical assistance and 22% receive fertility treatments. A half million assisted reproductive therapy (ART) cycles are performed annually in Europe (De Mouzon et al., 2010) and expected to rise within the next decade as the prevalence of infertility climbs (due to maternal age and male infertility). In humans, however, most pregnancy losses occur before or during embryo implantation (Teklenburg et al., 2010). In 2012, in France only 19% of patients involved in IVF/ICSI delivered despite transfer of a mean of 2 embryos (FIVNAT, 2012). The main limiting factor to successful live births remains embryo implantation, described by R.G. Edwards as "the last barrier" in reproductive medicine (Edwards, 2006). Classical solutions developed to overcome this high fail-

Abbreviation: LBR, Live-birth rate; ART, assisted reproductive therapy; ET, embryo transfer; RIF, repeated embryo implantation failure; IL, Interleukin; uNK cells, uterine Natural Killer cells; hCG, human chorionic gonadotrophin.

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ure rate aim to improve embryo quality and select the best one to transfer or to improve uterine-embryo synchronization. As complementary innovative approach, we propose to focus on optimizing the immune dialogue between the embryo and mother at implantation. Pre-implantation endometrial immune remodeling is vital for pregnancy, to prepare future maternal immune tolerance, protect the fetus, and regulate placentation. A local immunity switch from the adaptive (Th1) to the innate (Th2) type (Gellersen et al., 2007) occurs during the implantation window; after this switch, most endometrial immune cells are uterine natural killer (uNK) cells. This transient immune switch, together with adequate uNK cell activation, appears fundamental in enabling local maternal tolerance and thus fetal survival. uNK cells play an important role in building a healthy placenta by inducing local secretion of angiogenic factors by endometrial cells and modifying the structure of the pre-existing spiral arteries(Ashkar et al., 2003; Hanna et al., 2006). uNK cells are very different from circulating NK cells — by their phenotype, their repertoire of activating and inhibiting receptors, the cytokines they secrete, and their low cytotoxic potential. In physiological conditions, uNK cells are not spontaneously cytotoxic; rather, their main biological functions are to produce angiogenic and immunotrophic cytokines (IL-18, TGF beta, IL-10, etc.), which promote placental growth. However, in a Th1-dominant environment, uNK become killer cells able to recognize trophoblastic cells as non-self and reject them. Moreover, the uNK cells are not alone in the endometrium: in a high Th1 environment, dendritic and Treg cells initially helper cells that may also become real killers (Hanna and Mandelboim 2007; Blois et al., 2011). An orchestrated, balanced local immune biological reaction is required during the mid-luteal phase to enable the active step of embryo attachment but also to regulate the invasion phase.

We previously observed that the endometrium of women with repeated embryo implantation failures (RIF) contains both abnormal levels of uNK cells (too few or too many) and dysregulated expression of IL-15 (involved in uNK-cell maturation) and IL-18 (involved in the essential angiogenic process) compared with fertile women (Ledee-Bataille et al., 2004, 2005; Petitbarat et al., 2011).

To document the uterine immune environment, we therefore defined a specific combination of biomarkers, including the ratios of endometrial IL-18/TWEAK mRNA and IL-15/Fn-14 mRNA and the $CD56^+$ cell count. The IL-18/TWEAK mRNA ratio is used to document the local cytotoxic/angiogenic equilibrium and the IL-15/Fn-14 mRNA ratio to assess the state of activation and maturation of uNK cells, while the $CD56^+$ cell count simultaneously measures their mobilization. IL-15 is directly involved in post-ovulatory recruitment and maturation of uNK cells in the uterus (Kitaya et al., 2005) and, under their control, is essential for adequate Th2 cytokine production (Eriksson et al., 2004). IL-18 is a Th2-promoting cytokine that, through the action of angiopoietin-2, plays a role in the destabilization of spiral arteries (Goldman-Wohl et al., 2000; Croy et al., 2003) but become Th1 and deleterious in excess. TWEAK and its ligand, Fn-14, act as immune regulators of the local Th1/Th2 cytokine balance in the human endometrium (Petitbarat et al., 2009). Some inadequate immune events may explain some cases of RIF (PCT/EP2013/065355). In a previous uncontrolled large RIF cohort study, we recently reported a uterine immune dysregulation rate of 82% (Ledee et al., 2016). With care personalization applied to counteract the observed uterine immune deregulation, the subsequent live-birth rate was excellent (40%), given their history (Ledee et al., 2016). This cohort study, however, was observational with pregnancy outcome after the subsequent embryo transfer (ET) collected from multiple physicians and ART units and no control group with comparable characteristics for oocytes, sperm, and transferred embryos.

The present one-to-one matched cohort study took place in a single ART unit (Hospital Les Bluets-Drouot, Paris) in 2012–2014.

Table 1
Clinical profile of women in the analyzed and non-analyzed groups according to the matching criteria.

	Analyzed group	Non-analyzed Group
Age category (pairing criteria)		
Less than 35 Y	52	52
from 35 to 38 Y	78	78
from 39 to 40	32	32
more than 41 Y	31	31
ART method (pairing criteria)		
IVF	24	24
ICSI with partner sperm	143	143
ICSI with donor sperm	2	2
ICSI with frozen testicular sperm	1	1
Frozen embryo transfer	23	23
Category of mature oocyte collected (pairing criteria)		
Less than 4 mature oocytes retrieved	62	61
From 5 to 9 mature oocytes retrieved	74	78
From 10 to 15 mature oocytes retrieved	29	27
More than 15 mature oocytes retrieved	5	4
Stage of embryo transferred (pairing criteria) (fresh transfer)		
Day 2 or 3	132	131
Day 5 or 6	35	36
double transfer Day 2/3 and Day 5/6	3	3
Number of embryos transferred (fresh transfer)		
1 embryo transferred	46	41
2 embryos transferred	91	105
3 embryos transferred	33	24
Stage of embryo transferred (pairing criteria) (frozen-thawed transfer)		
Day 3	3	3
Day 5 or 6	20	20
Number of embryos transferred (pairing criteria) (frozen-thawed transfer)		
1 embryo transferred	14	14
2 embryos transferred	9	9

The analyzed group comprised 193 women with a uterine immune profile before ET, and the non-analyzed group 193 matched patients without a uterine immune profile before their ET. To quantify the effect of immune profiling on uterine receptivity, each analyzed women was paired one to-one-to the closest corresponding non-analyzed women to ensure comparable embryo quality (maternal age, number of mature oocytes collected, method of fertilization, stage and number of embryos transferred).

2. Materials and method

2.1. Protocol approval and patient consent

The Institutional Review Board of St Louis Hospital (2011-A00994-37) approved this study.

All patients included in the ART program gave them inform consent before any fertility treatment (IVF/ICSI/Frozen ET). Patient undergoing an endometrial biopsy provided their additional written inform consent allowing uterine immune analysis.

2.2. Patients

This retrospective study included 386 women enrolled in our IVF/ICSI program (assisted reproductive unit of Les Bluets-Drouot, Paris) in 2012–2014. Table 1 describes the patients' demographic, clinical, and ART characteristics (including for matching variables).

The non-analyzed group is composed of 193 women treated with a conventional IVF/ICSI program. The analyzed group com-

Table 2
Causes of infertility, history of ART, and main indicators related to the treatment applied before the embryo transfer to women in the analyzed and non-analyzed groups.

	Analyzed Group	Non-analyzed Group	*P* value
Maternal age (years)	36.5 ± 3.6 (28–43)	36.6 ± 3.9 (26–44)	0.64 (pairing criteria)
BMI (kg/m^2)	22.7 ± 3.7 (16.3–37.9)	24.2 ± 4.69 (17.63–41.26)	0.01
Tobacco use (%)	21%	16%	0.78
FSH (IU) on Day-3	7.52 ± 2.95 (1.6–19.7)	7.35 ± 2.63 (1.7–17.9)	0.56
LH (IU) on Day-3	5.75 ± 3.30 (0.6–34)	5.72 ± 2.64 (0.5–17.6)	0.98
Oestradiol (pg/ml) on Day-3	48 ± 29 (2.7–188)	47 ± 28 (7–141)	0.11
AMH (ng/ml) on day-3	2.85 ± 2.60 (0.1–17.5)	2.95 ± 2.6 (0.1–15.3)	0.79
Primary or secondary infertility	100 primary/93 secondary	101 Primary/92 secondary	1
Main etiology of infertility or reason of ART			0.32
Tubal infertility	29	26	
Endometriosis	6	12	
Recurrent miscarriages	6	1	
PCO	8	7	
Idiopathic infertility	10	10	
Ovariann insufficiency	17	12	
Male infertility (AOTS, cryptozoospermia, azoospermia)	64	78	
Male and female infertility	53	47	
History of infertility treatment			
Previous ICSI or IVF attempt	2.5 ± 1.69 (0–10)	1.29 ± 1.55 (0–8)	<0.001
Number of previous embryos replaced	5.9 ± 4.25 (0–25)	2.5 ± 3.3 (0–21)	<0.001
Duration of infertility	5.6 ± 3.4 (1–21)	4.8 ± 3.2 (1–17)	0.01
Obstetrical history			
Gravidity	1.22 ± 1.32 (0–7)	1.03 ± 1.31 (0–7)	0.18
Parity	0.31 ± 0.53 (0–2)	0.37 ± 0.67 (0–3)	0.34
Spontaneous abortion	0.64 ± 1.14 (0–7)	0.39 ± 0.81 (0–7)	0.038
Protocol of ovarian stimulation			
Antagonist protocol	105	100	0.75
Agonist protocol	65	70	0.75
Natural cycle (frozen ET)	10	12	0.75
Substitued cycle (frozen ET)	13	11	0.75
Initial dose of gonadotropins (IU)	230 ± 83 (60–450)	227 ± 83.7 (75–450)	0.57
Total dose of gonadotropins (IU)	2157 ± 987 (420–5400)	2166 ± 978 (600–4800)	0.93
Number of oocytes collected	8.2 ± 4.77 (1–31)	8.3 ± 4.98 (1–28)	0.85
Number of matures oocytes	6.37 ± 3.88 (1–25)	6.23 ± 3.60 (1–20)	0.8 (pairing criteria)
Total number of embryos obtained	5 ± 3.5 (1–22)	5 ± 3 (1–19)	0.92
Number of embryos transferred	1.87 ± 0.68 (1–3)	1.85 ± 0.62 (1–3)	0.38

Statistical analyses used an Anova test for continuous data or a chi-2 test for categorical data. Means are presented with standard deviation (±) and range between brackets.

prises 193 women who had a uterine immune profile and then underwent IVF/ICSI with care personalized according to the results of the profile (for woman with immune dysregulation). Women had been referred by their physicians for this profile for various reasons (Table 2): unexplained implantation failure of previously transferred embryos for 136, unexplained infertility for 13, unexplained recurrent miscarriages for 14, to optimize the ET for 21 women with severe ovarian insufficiency, and before ART with no previous failure, miscarriage, or ovarian insufficiency for only 9 patients.

All women in the analyzed group underwent fresh or frozen ET within 9 months of the endometrial evaluation. Only the first ET after the evaluation was considered.

If the profile results showed immune dysregulation (over- or low activation of immune regulation), the subsequent uterine preparation was personalized to attempt to optimize subsequent embryo implantation.

2.3. Matching process

To document the effectiveness of uterine immune profiling on outcome while trying to control the part dedicated to oocyte and embryo quality, each analyzed patient was matched at the time of the ET with the closest patient enrolled in our ART program who met the following criteria:

- Same age category: <35 years old, 35–38, 39–40, 41 or older

If fresh ET was performed:

- Same method of fertilization: IVF, ICSI with partner sperm, ICSI with donor sperm, ICSI with frozen testicular sperm
- Same category of mature oocytes: <4 mature oocytes, 5–9 mature oocytes, 10–15 mature oocytes, >15 mature oocytes. If the same category was not available, a difference of only 1 mature oocyte was accepted for the matching process.
- If possible, the same embryo stage (day 2/3 or day 5/6) and number of embryos transferred. We accepted 2 embryos on day 2/3 as a match for a fresh transfer for 1 embryo day 5 (but not the opposite).

If frozen-thawed ET was performed, the transfers had to involve the same number of embryos at the same stage.

2.4. Determination of uterine immune profile

Women in the analyzed group had undergone uterine immune profiling, as previously described in detail (Ledee et al., 2016). Briefly, after histological dating of an endometrial biopsy sample to confirm the mid-luteal phase, RNA was extracted with the RNeasy Plus kit (Qiagen, Courtabeuf, France), according to the manufacturer's instructions. The RNA was reverse-transcribed into cDNA with the first-strand cDNA synthesis kit for RT-PCR (Roche Diagnostic, Meylan, France). IL-15/Fn-14 and IL-18/TWEAK mRNA ratios were determined by quantitative RT-PCR with the Light Cycler 480

SYBR Green I Master mix (Roche Diagnostic), and uNK cells were counted after CD56+ immunohistochemistry.

The uterine immune profiling is defined by the association of three biomarkers and the norm was defined in a fertile cohort (Ledee et al., 2016):

- The IL-18/TWEAK mRNA ratio, which reflects the Th1/Th2 balance and local angiogenesis. An IL-18/TWEAK mRNA ratio was considered low when below 0.03 (mean minus 1 SD) and high when greater than 0.12 (mean + 1 SD). In a previous study, the latter cutoff (>0.12) was also associated with activation of the uNK cytotoxic receptor NKp46, which attested to the transformation of uNK cells into cytotoxic killer cells (Petitbarat et al., 2011).the IL-15/Fn-14 mRNA ratio, which reflects uNK cell maturation. An IL-15/Fn-14 mRNA ratio was considered low when below 0.3 and high when greater than 3. A low IL-15/Fn-14 reflect the immaturity of uNK cells while a high ratio suggests an over-immune activation.
- The number of CD6 positive cells: the $CD56^+$ cell count was therefore considered low when below 10 and high when greater than 100.

To establish the endometrial immune profile, a step-by-step procedure first considered the IL-18/TWEAK mRNA ratio (reflecting local angiogenesis and possibly a Th-1 deviation), then the $CD56^+$ cell count (reflecting uNK cell mobilization), and finally the IL-15/Fn-14 mRNA ratio (indicative of uNK cell maturation and uNK cytotoxic activation).

2.5. Patients with personalized care in the analyzed group

All patients with immune dysregulation according to the immune profile had personalized care. Modification of treatments applied were function of the identified immune dysregulation diagnosed.

2.5.1. Modification of treatment for women with immune over-activation

As previously detailed (Ledee et al., 2016), immune over-activation was characterized by high ratios of IL-18/TWEAK mRNA suggesting excess of Th-1 cytokines and/or high ratios of IL-15/Fn-14 mRNA suggesting uNK activation in Killer cells, and/or CD56+ cell count over 100/field suggesting an over uNK cells mobilization.

These women received specific luteal hormonal support: high daily vaginal doses of progesterone (1200 mg) (compared with the standard 600 mg) (Szekeres-Bartho et al., 1997, 2009) and oral estradiol supplementation (6 mg) (Ledee et al., 2006). Hormonal support began on the day of oocyte retrieval and continued through 8 weeks after ET for pregnant women. We recommended that women with this profile avoid sexual intercourse after ET until the pregnancy test. As a first line of treatment, women received also 20 mg of corticoids (Elenkov 2004; Moustaki et al., 2011; Eddy et al., 2014) and vitamin E (an antioxidant, I g daily) from day 3 of ovarian stimulation until the pregnancy test.

If in their past IVF/ICSI history, women who had not become pregnant with corticoids before the endometrial immune profile, a single slow perfusion of 4% diluted Intralipid® (Fresenius-Kabi) was proposed on days 8–10 of the ET cycle (Roussev et al., 2008).

If pregnancy occurred

- corticotherapy was continued at full dose until 8 weeks after ET, then decreased slightly, and finally stopped at 10 weeks.
- If Intralipid® was administered; another slow perfusion was administered at 3 weeks after ET and again at 7 weeks afterwards.

2.5.2. Modification of treatment for women in the group with low endometrial immune activation levels

A low immune activation profile was characterized by low IL-15/Fn-14 mRNA ratio (reflecting immature uNK cells) or a very low local IL-18/TWEAK mRNA ratio (Ledee et al., 2016) and/or the absence of their mobilization (CD56 + cell count <10).

Endometrial scratching or other local injury was performed in the mid-luteal phase of the cycle before ET to stimulate subsequent expression of adhesion molecules (Gnainsky et al., 2010). We therefore recommended supplementing the luteal phase with human chorionic gonadotrophin (hCG) for the positive reported effect on local uNK activation and to promote local angiogenesis (Perrier D'hauterive et al., 2007; Kane et al., 2009). It was subcutaneously administered at 4, 6, and 8 days after oocyte retrieval or the start of progesterone support, that is, during the implantation window.

Sexual intercourse after the ET was also recommended (Robertson, 2005).

These patients received a standard luteal support with 800 mg of progesterone administrated vaginally.

2.6. Patients with conventional care

Women in the analyzed group without uterine dysregulation and all women in the non-analyzed group underwent a standard IVF/ICSI program, without either local injury before the ET cycle or supplementation with corticoids/Intralipid® or hCG.

They all received a standard luteal support with 800 mg of progesterone administrated vaginally.

2.7. Statistical analysis

We compared the analyzed and non-analyzed groups as well as the dysregulated-treated versus control groups with an Anova test for continuous data, and a Chi-square test for categorical data. A P value below 0.05 was considered significant.

Patients with a positive β-hCG below 100 IU were considered as not pregnant. Initiated pregnancy was defined by a positive β-hCG test over 100 IU. Clinical pregnancy was defined by the visualization of a yolk sac at 4 weeks of gestation. Ongoing pregnancy was defined by the visualization of yolk sac with cardiac activity at 10 weeks of gestation. The implantation rate at 4 weeks of gestation was defined as the ratio of observed yolk sacs at 4 weeks of gestation to the total number of embryo transferred. The ongoing implantation rate at 10 weeks of gestation was defined as the ratio of embryo with cardiac activity observed by ultrasound at 10 weeks to the total number of embryos transferred. The implantation rate at birth was defined as ratio of the number of babies born after 26 weeks to the total number of embryo transferred.

The total live birth rate was defined as the ratio of the number of birth per patient to the number of embryo transfer performed irrespectively of the number of embryo replaced.

The miscarriage rate was defined as the ratio of the number of initiated pregnancies (with a positive test over 100 IU) lost through 10 weeks of gestation to the number of initiated pregnancies.

3. Results

3.1. Clinical characteristics and type of ART in the analyzed and non-analyzed groups

Table 2 details for each group the causes of infertility, ART history, basal ovarian reserve, and main characteristics of the ART used for this ET. The women's mean age was 36.6 years. The groups did not differ for the causes of infertility or for tobacco use. Although mean BMI was significantly higher in the non-analyzed group (24.2

Table 3
Pregnancy outcome in the analyzed and non-analyzed groups.

Pregnancy outcome	Analyzed Group	Non-analyzed Group	*P* value
Implantation rate at 4 weeks of gestation (%)	23.8 ± 4 (0–100)	18.3 ± 3 (0–100)	0.18
Implantation rate at 10 weeks of gestation (%)	19.43 ± 3 (0–100)	11.66 ± 3 (0–100)	0.03
Implantation rate at birth (%)	19.43 ± 4 (0–100)	11.14 ± 3 (0–100)	0.01
Miscarriage per initiated pregnancy (%)	17 ± 4 (0–100)	41 ± 5 (0–100)	0.003
Total Live birth rate (%)	27.5 ± 4 (0–100)	16.6 ± 4 (0–100)	0.01
Number of Live birth	52	29	0.68
Number of Singleton	45	26	0.95
Number of Twins	7	3	0.95
Term of birth (weeks of amenorrhea)	38.7 ± 2 (26.5–41.5)	39 ± 1.7 (33.5–41.5)	0.44
Weight of birth (Kg)	3.320 ± 0.580 (0.900–4.900)	3.196 ± 0.601 (2.170–4.600)	0.77

Means are presented with standard deviation (±) and range between brackets.

compared with 22.7 in the analyzed group, $P = 0.01$), BMI was in the normal range in both groups. No difference was observed for basal hormonal levels (FSH, LH, E2, AMH) on day-3, ovarian stimulation protocols, or initial and total gonadotropin doses administered during ovarian stimulation. The mean number of embryos per woman produced after IVF/ICSI was also similar in the two groups (5 embryos).

Beyond the pairing criteria, the two groups appeared to be comparable according to the main parameters related to the ovarian response and oocyte/embryo quality.

As expected, the history of infertility was different. The number of previous attempts and of previously transferred embryos as well as the duration of infertility and number of spontaneous abortions were significantly higher in the analyzed group. Most women who had an endometrial immune profile were referred for this investigation precisely because of their history of unexplained implantation failures, with or without previous miscarriages.

3.2. Pregnancy outcome in the analyzed and non-analyzed groups

Table 3 summarizes the pregnancy outcomes in each group.

Although the implantation rate at 4 weeks of gestation did not differ significantly between the two groups, the implantation rates at 10 weeks and the total birth rates differed significantly. The implantation rate at birth (number of baby born/number of embryo transferred) was 19.4% in the analyzed group, compared with 11.1% in non-analyzed group ($P = 0.01$). The most impressive finding, however, was the drastic difference in the miscarriage rate per initiated pregnancy: 17% in the analyzed group versus 41% in the non-analyzed group ($P = 0.003$).

Consequently, the LBR in the analyzed group was significantly higher than in the non-analyzed group (27.5% versus 16.6%, $P = 0.01$) despite the fact that the analyzed group had a longer duration of infertility, more previous IVF/ICSI attempts, and more previously transferred embryos that failed to implant.

The groups did not differ concerning term or weight at birth.

3.3. Pregnancy outcomes according to endometrial immune profile

The women in the analyzed group were divided into 2 subgroups according to their endometrial immune profiling:

- 151 with uterine immune-dysregulation (78.3%)
- 42 with no local immune dysregulation (21.7%)

3.3.1. Patient with immune dysregulation (Table 4)

For patients with immune dysregulation and therefore care personalization, LBR was twice higher than observed in the matched control group with conventional cares (30.46% versus 16.56%, OR: 2.2 [1.27–3.83] $P = 0.004$). Implantation rates at 10 weeks of gestation was significantly higher than observed in corresponding matched-control group (21.6% versus 12.8%, OR = 2.48 [1.7–4.18] $P = 0.025$) with a significant reduction of miscarriages at the first trimester (17.9% versus 43.2%, OR: 0.28 [0.11–0.7], $P = 0.005$).

Regarding dysregulated patients, 55% (107/193) showed an over-immune activation and 23% (44/193) show the opposite mechanism a low immune local activation.

Patient with immune over-activation did not differ significantly from their matched controls for implantation, ongoing pregnancy, or total birth rates, but their miscarriage rate per initiated pregnancy was significantly lower than that in the control group (21.6% versus 43.6%, $P = 0.042$). The LBR consequently tended to be higher than observed in their matched controls (27.1% versus 20.56% in the controls, $P = 0.26$) (data not shown).

For patient with low immune uterine activation, implantation rate at 4 week, 10 week of gestation and at birth were all significantly higher than in corresponding the control group (30.3%, 25.76%, and 25.76% respectively versus 9.09%, 7.14%, and 5.68%, $P = 0.004$, $P = 0.01$, $P = 0.002$). The miscarriage rate per initiated pregnancy did not differ significantly, however, although there was a trend toward a lower rate in the analyzed women ($P = 0.12$). The LBR in this group with low immune activation was 38.6% compared to 6.8% in the control group ($P < 0.001$) (data not shown).

Patients with dysregulation received a personalized treatment according to their respecting immune profile (over immune activation or low activation) to control their immune disorder. We therefore postulated that the significant difference of live birth observed between dysregulated patients and controls was the direct consequence of the care personalization.

3.3.2. Patients with no dysregulation (Table 5)

The analyzed patients with no dysregulation did not differ from their matched controls for implantation rate at 4 weeks of gestation, at 10 weeks of gestation or at birth as well as for miscarriage rates at the first trimester. Beyond the matching criteria, this analyzed group and its control group did not differ either for other criteria as basal ovarian reserve, initial and total gonadotropin doses, protocols, total embryos obtained, quality of transferred embryos. These results confirm there's no effect of the diagnostic biopsy itself, realized (at least two months before ET) for immune profiling on the pregnancy outcome.

3.4. Pregnancy outcome by age group in the analyzed and non-analyzed women

Table 6 illustrates the live birth and miscarriage rates according to age in dysregulated and corresponding control groups for patients younger or older than 39 years old. Among women younger than 39 years with immune dysregulation and subsequent personalized care, 38.61% gave birth to live babies, compared with 21.78% of their matched controls ($P = 0.009$); they also had a drastic lower

Table 4
Pregnancy outcome of analyzed patients with immune dysregulation.

Pregnancy outcome	Group with uterine immune dysregulation	Correspondingnon-analyzed group	odds ratio (95% CI)	*P* value
Number of patients (%)	151 (78.3%)	151 (78.3%)		
Mean Age	36.6 ± 3.6 (28–43)	36.5 ± 4 (26–44)		0.79
Mean number of oocytes collected	8.20 ± 4.37 (1–23)	8.22 ± 5 (1–26)		0.91
Mean number of embryos transferred	1.88 ± 0.85 (1–3)	1.82 ± 0.66 (1–3)		0.51
Implantation rate at 4 weeks of gestation (%)	26.49 ± 4 (0–100)	19.98 ± 3.6 (0–100)	1.81 (1.12 to 2.9)	0.12
Implantation rate at 10 weeks of gestation (%)	21.63 ± 3.5 (0–100)	12.80 ± 3.2 (0–100)	2.48 (1.47 to 4.18)	0.025
Implantation rate at birth (%)	21.63 ± 3.5 (0–100)	12.14 ± 3.1 (0–100)	2.65 (1.55 to 4.54)	0.015
Miscarriage per initiated pregnancy (%)	17.86 ± 3.5 (0–100)	43.18 ± 5 (0–100)	0.286 (0.115 to 0.71)	0.005
Total Live birth rate (%)	30.46 ± 4.5 (0–100)	16.56 ± 4 (0–100)	2.2 (1.27 to 3.83)	0.004

Means are presented with standard deviation (±) and range between brackets.

Table 5
Pregnancy outcome of analyzed patients with no immune dysregulation.

Pregnancy outcome	Group with no uterine immune dysregulation	Correspondingnon-analyzed group	Odds ratio (95% CI)	*P* value
Number of patients (%)	42 (21.7%)	42 (21.7%)		
Mean Age (years)	36.7 ± 3.3 (29–43)	37.1 ± 3.64 (29–43)		0.59
Mean number of oocytes collected	8.5 ± 3.65 (1–16)	9.1 ± 4.8 (3–28)		0.47
Mean number of embryos transferred	2 ± 1 (1–3)	1.9 ± 0.67 (1–3)		0.62
Implantation rate at 4weeks of gestation (%)	13.9 ± 3 (0–100)	19.11 ± 3 (0–100)	0.81 (0.22 to 2.8)	0.37
Implantation rate at 10 weeks of gestation (%)	11.51 ± 3 (0–100)	14.23 ± 3 (0–100)	1.28 (0.32 to 5.15)	0.44
Implantation rate at birth (%)	11.51 ± 3 (0–100)	14.23 ± 3 (0–100)	1.28 (0.32 to 5.15)	0.43
Miscarriage per initiated pregnancy (%)	12.5 ± 3 (0–100)	27.27 ± 4 (0–100)	0.28 (0.02 to 3.5)	0.68
Total Live birth rate (%)	16.67 ± 4 (0–100)	19.51 ± 4 (0–100)	0.85 (0.28 to 2.6)	0.33

Means are presented with standard deviation (±) and range between brackets.

Table 6
Pregnancy outcome in the analyzed and non-analyzed women below and after 39 years old.

	Less than 39 years			Over 39 years		
	Group with uterine immune dysregulation	Corresponding non-analyzed group	*P* value	Group with uterine immune dysregulation	Corresponding non-analyzed group	*P* value
Miscarriage per initiated pregnancy%	11.36% (44)[b]	39.47% (38)[d]	0.003	41.67% (12)[b]	66.7% (6)[d]	0.34
Total Live birth rate%	38.61% (101)[a]	21.78% (101)[c]	0.009	13.73% (50)[a]	6% (50)[c]	0.19

[a] $P = 0.002$.
[b] $P = 0.015$.
[c] $P = 0.014$.
[d] $P = 0.22$.

miscarriage rate per initiated pregnancy (11.36% versus 39.47%, $P = 0.003$). As always, as maternal age increased, subsequent LBR decreased and the miscarriage rate rose. Only 13.73% of the women 39 years or older with dysregulation and personalized cares had live births with 41.67% of miscarriages versus 6% of birth and 66.7% of miscarriages in the matched control group ($P = 0.19$ and $P = 0.34$).

4. Discussion

Here we have described a one-to-one matched cohort study demonstrating the promise of assessing uterine receptivity from an immune point of view as a tool for increasing LBR in IVF/ICSI through personalized cares. We compared the pregnancy outcome of two groups of 193 patients enrolled in our IVF program. One was the analyzed group, all of whom had an endometrial immune profile that enabled personalized IVF/ICSI treatment for women with either form of immune dysregulation (over-activation or low immune activation) and the other the non-analyzed group, which had no immune profile and underwent conventional IVF/ICSI treatment. The matching process for the two groups sought to neutralize the variables linked to either embryos quality or maternal age that were likely to confound the analysis. The biological environment was the same. Matching criteria included maternal age, number of matured oocytes, stage and number of embryos transferred. The groups did not differ for the women's basal hormonal levels, response to hormonal stimulation, or ovarian stimulation protocols applied. Although the case women had a much more severe history of infertility, their LBR after immune assessment and treatment was 85% higher than that of their matched controls: 30% versus 16%. This higher LBR appears to be related to a significantly lower miscarriage rate during the first trimester. This result suggests that immune profiling and subsequent personalized treatment enables a positive effect on the uterine side, although it could not correct the reduced ovarian quality related to maternal aging.

These results provide further evidence that immune preparation of the uterus is required to receive the embryo and start its dialogue with the embryo. That is, generating good quality embryos is an absolute necessity for live births, but so is their transfer into a receptive uterus. Human implantation may be described simply as a three-step process starting with apposition and followed by attachment — adhesion of a competent blastocyst to the endometrial epithelium. Attachment requires active local endometrial reactivity on the maternal side. The third step is extensive invasion of a receptive endometrium by the trophoblast cells covering the blastocyst, successful only if the adhesion step is followed immediately by an anti-inflammatory reaction enabling the local tolerance required for effective invasion.

In this study, uterine immune disorders affected 78.3% of the analyzed group: 55.4% had immune over-activation and 22.8% low immune activation. This distribution is similar to that observed in

another cohort of 394 women with RIF (Ledee et al., 2016). The improvement of LBR in women with endometrial immune dysregulation receiving personalized care to normalize their immune profile highlights the importance of the local immune equilibrium for pregnancy and the value of the uterine immune receptivity.

Different immune mechanisms may be involved in and generate uterine immune disorders. For patients with immune over-activation, application of personalized care significantly decreased the first-trimester miscarriage rate. Such observation suggests the treatment had a positive effect at the time of invasion. Nevertheless, distinct immune mechanisms may cause deleterious uterine immune over-activation.

In a Th1-dominant environment, uNK cells become killer cells able to recognize trophoblastic cells as non-self and hence to reject them as well as dendritic cells and T regulatory T cells. (Hanna and Mandelboim 2007; Blois et al., 2011). Dendritic cells in particular may differentiate into deleterious DC-1 cells rather than the more helpful DC-2 cells (Tirado-Gonzalez et al., 2012), and T cells may differentiate into deleterious Th17 lymphocytes rather than into the Treg cells required for pregnancy (Sharma, 2014). Uterine immune over-activation may also result in local hyper-activation of complement through the mannose-binding lectin pathway, as recently suggested in a murine abortive model (Petitbarat et al., 2015). In theory, each specific mechanism inducing immune over-activation requires a specific, targeted treatment (Coulam and Acacio, 2012). Limitations of study design is related to the fact that distinct therapies have been used to control the over-immune activation, sometimes simply based on history and not on the observed normalization of the immune profile under the tested therapy. We may therefore assume that the fairly simplistic approach we applied here of adding corticoids or (if corticoids previously failed) Intralipid® for women with immune over-activation can be improved. Direct observation of the normalization of an immune profile under the therapy tested represents the optimal approach. In our previous cohort study, we reported that corticoids could normalize endometrial immune biomarkers in only 54% of over-activated uterine immune profile (Ledee et al., 2016). However, this observation was made on a small number of patients and need to be confirmed on a larger cohort. Better understanding on precise mechanisms of action of either corticoids or Intralipid® on deregulated immune uterine profiles are required.

The profile of low uterine immune activation in the analyzed group (characterized by the uNK cell immaturity or lack of mobilization) revealed disruption of the molecular mechanisms involved in effective adhesion and local angiogenesis. Personalized care therefore aimed to stimulate immune cell maturation and adhesion molecule mobilization and local expression. The high LBR observed (38.64%) among the 44 women for whom we diagnosed low local activation, with the high rate of implantation (30.3%) as early as 4 weeks suggesting an early positive impact. uNK cells play an important role in building a healthy placenta by inducing endometrial cells to secrete angiogenic factors locally and by modifying the structure of the pre-existing spiral arteries (Ashkar et al., 2003; Hanna et al., 2006).

Based on this one-to-one matched cohort study, a clinical trial named PRECONCEPTIO and designed as a prospective randomized controlled study (NCT-02262117) is ongoing. Objective is to evaluate the interest of care personalization based on pre-conceptional endometrial immune profiling as an innovative tool to increase birth rates in reproductive medicine. Patients with immune deregulation according to our defined biomarkers will be randomized between conventional versus personalized IVF/ICSI cycles. The outcome of the study will be the live birth rate at the first subsequent embryo fresh transfer.

5. Conclusion

Uterine immune profiling enables an integrated approach of infertility that includes endometrial immunity as a key factor in planning personalized IVF/ICSI treatments. Endometrial immune profiling seems effective in determining whether a woman's uterus is immunologically ready to accept an embryo and, if not, what specific immune mechanisms are involved for each woman. This diagnosis may then lead physicians to an effective individual understanding of the immune profile of uterine receptivity so that they can provide appropriate treatment and advice that optimize the conditions of uterine receptivity. Personalization of treatment according to the woman's uterine immune balance produced a very significantly higher live birth rate but randomized prospective study is required to prove the hypothesis.

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