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(54) Benevnelse **WHARTON'S JELLY MESENCHYMAL STEM CELLS FOR USE FOR TREATING SEPSIS**

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MESENCHYMAL STEM CELLS, THE PREPARATION METHOD THEREOF AND THE USES THEREOF

FIELD OF THE INVENTION

The present invention relates to human mesenchymal stem cells derived from Wharton's jelly, their preparation method and their therapeutic uses.

PRIOR ART

Although often misunderstood, septic shock represents, along with myocardial infarction, the eleventh cause of death throughout the world. As the initial cause of admission and death in non-coronary resuscitation units, its incidence, ranging between 50 and 100 cases for 100,000 inhabitants, has continually grown in recent years (Dombrovskiy *et al.*, 2007). Increasing life expectancy, the growing number of multi-resistant bacteria and a more commonly made diagnosis are the source of this phenomenon.

The physiopathology of septic shock is particularly complex (Iskander *et al.*, 2013) and simultaneously involves both pro- and anti-inflammatory cytokines, as well as immunoparalysis. There is no specific treatment for septic shock due to the inability of conventional pharmaceutical molecules to act on both the inflammatory aspect and the anti-inflammatory aspect, while restarting the immune system.

However, many studies using murine models of endotoxemia or peritonitis have demonstrated the ability of mesenchymal stem cells (MSCs) to decrease plasma levels of IL-6, IL-1β, IL-12, IL-2 and IL-17 (Chao *et al.*, 2014a; Kim *et al.*, 2014; Luo *et al.*, 2014; Pedrazza *et al.*, 2014) and lower the tissue concentration of TNFα, IL-6, IL-1β, IL-12 at the pulmonary, liver and intestinal level (Gonzalez-Rey *et al.*, 2009), or at the bronchoalveolar fluid level (Mei *et al.*, 2010a).

The ability of MSCs to limit the tissue migration of neutrophils results in the moderation of the harmful effects of inflammation on tissues (Rojas *et al.*, 2014). Studies of the histological sections of mice subjected to polymicrobial sepsis by ligation and perforation of the caecum (CLP) or subjected to sepsis by endotoxemia demonstrate a reduction in the inflammation of lung and kidney tissues when they are treated with MSCs (Krasnodembskaya *et al.*, 2010). Similarly, these mice have biological markers of the functions of organs such as amylases, creatinemia, bilirubin, aspartate aminotransferases (ASAT) and alanine aminotransferases (ALAT) that are significantly better than mice treated with a saline solution (Luo *et al.*, 2014; Pedrazza *et al.*, 2014).

In addition to their ability to limit tissue alteration, MSCs are capable of increasing bacterial clearance during septic shock. Indeed, several teams have demonstrated that injecting MSC into septic mice resulted in a reduction of bacteremia by increasing the phagocytic activity of monocytes, macrophages and neutrophils, but also by synthesis and secretion of antibacterial peptides by the MSCs themselves, such as LL-37 and hepcidin (Alcayaga-Mircoanda *et al.*, 2015; Devaney *et al.*, 2015; Gonzalez-Rey *et al.*, 2009; Hall *et al.*, 2013; Krasnodembskaya *et al.*, 2010; Mei *et al.*, 2010b).

Furthermore, MSCs have intrinsic "homing" properties, which give them a susceptibility to migrate, according to a chemokines gradient, towards the injured organs, such as the liver, the kidneys and the lungs. This ability to join the damaged tissues is a particularly beneficial attribute of MSCs in the context of an organ failure pathology such as septic shock (Wannemuehler *et al.*, 2012).

Finally, the reduction in tissue damage by injecting MSCs in murine models with severe sepsis results in an increase in survival. Numerous studies demonstrate a significant reduction in the death of animals treated pre- or post-sepsis using MSCs (Alcayaga-Miranda *et al.*, 2015; Devaney *et al.*, 2015; Gonzalez-Rey *et al.*, 2009; Krasnodembskaya *et al.*, 2012; Luo *et al.*, 2014; Németh *et al.*, 2009). Thus, due to their abilities to regulate hyper-inflammation and to moderate tissue damage, MSCs appear to be able to significantly improve post-septic shock survival.

A distinction is made between three major sources of MSCs: bone marrow (BM), adipose tissue and Wharton's jelly (WJ) of the umbilical cord. Compared with MSCs of BM, MSCs of WJ obtained in a simple, non-invasive and anaesthesia-free manner, would allow the MSC donation to be democratized by overcoming any risks associated with the removal of BM. However, even though the MSCs of BM or the MSCs of adipose tissue have been studied in animal models of septic shock, the potential of MSCs derived from Wharton's jelly in this indication remains poorly documented.

To date, only 5 publications have analysed the therapeutic potential of WJ-MSCs on septic shock using a CLP murine model (Chao *et al.*, 2014a; Condor *et al.*, 2016; Wu *et al.*, 2015; Zhao *et al.*, 2014). However, the cells used in these studies are fresh cells. No study published to date relates to thawed WJ-MSCs in the indication of septic shock, this may be due to the general knowledge of the impact of freezing and thawing on the pharmacological and immunomodulatory potential of stem cells (François *et al.,* Cytotherapy. 2012.14(2):147-52; Chinnadurai *et al.,* 2016; Moll *et*

al., 2016).

Furthermore, Mezey and Nemeth (2015) have described a reduction in the antibacterial properties of MSCs when they are injected at a later stage.

Accordingly, to date these technical constraints prevent the use of WJ-MSCs in clinical conditions for therapeutic treatment.

Contrary to all expectations, the inventors of the present invention demonstrate for the first time that thawed WJ-MSCs preserve their therapeutic potential and can be used in the treatment of sepsis.

SUMMARY

The present invention relates to thawed human mesenchymal stem cells (MSCs) derived from Wharton's jelly for their use in the treatment of sepsis, in particular of septic shock, said thawed human MSCs derived from Wharton's jelly being characterized in that:
their expression level of CD44 is at least 20 % lower than the expression level of CD44 in fresh human MSCs derived from Wharton's jelly; or
their level of CD90, CD73, CD105, CD146, CD166 or CFU-F is at least 10 % lower than the level of CD90, CD73, CD105, CD146, or CD166, respectively, in fresh human MSCs derived from Wharton's jelly.

In one embodiment, the thawed human MSCs derived from Wharton's jelly of the present invention are characterized in that the expression level of at least one marker selected from CD90, CD73, CD105, CD29, CD146, CD166, HLA-ABC is at least 10 % lower than the expression level of the same marker in fresh human MSCs derived from Wharton's jelly.

In one embodiment, the thawed human MSCs derived from Wharton's jelly of the present invention are characterized in that said MSCs express at least one protein selected from among the group comprising ACTB, ANXA1, CAPZB, LASP1, PRDX2, PRDX3, PSA3, RS12 and SYWC.

In one embodiment, the thawed human MSCs derived from Wharton's jelly of the present invention are characterized in that said MSCs do not substantially express at least one protein selected from among the group comprising ACTS, AL1B1, ANX10, GBB1, GBB2, GPRIN1, DTNA, MIPO1, PSB3 and PSDE.

In one embodiment, the thawed human MSCs derived from Wharton's jelly of the present invention are characterized in that said MSCs, under *in vitro* conditions and/or under non-inflammatory conditions, secrete at least one growth factor selected from among BMP-7, IGFBP-1, insulin, FGF-7, NT-4 and VEGF-D.

In one embodiment, the thawed human MSCs derived from Wharton's jelly of the present invention are characterized in that said MSCs, under *in vivo* conditions and/or under inflammatory conditions, secrete at least one growth factor selected from among BMP-7 and TGFβ3.

In one embodiment, the thawed human MSCs derived from Wharton's jelly of the present invention are characterized in that said MSCs, under *in vivo* conditions and/or under inflammatory conditions, do not substantially secrete IGFBP-1.

In one embodiment, the thawed human MSCs derived from Wharton's jelly of the present invention are characterized in that said cells are derived from a human umbilical cord tissue from a mother who meets at least one of the following criteria: having received an administration of oxytocin during childbirth, having given birth by directed labour, having given birth at full term, not having presented pre-eclampsia during the pregnancy, whose child has not presented neonatal disorders and has been subject to an intake of tobacco smoke during the pregnancy.

In one embodiment, the thawed human MSCs derived from Wharton's jelly of the present invention are characterized in that said MSCs are clinical grade cells.

In one embodiment, the thawed human MSCs derived from Wharton's jelly of the present invention are characterized in that said cells are derived directly from thawing without reculturing after thawing.

The present invention also relates to a pharmaceutical composition comprising thawed human MSCs derived from Wharton's jelly according to the present invention and a pharmaceutically acceptable excipient, for use in the treatment of sepsis.

DEFINITIONS

In the present invention, the terms used hereafter are defined as follows:

"**Absence of neonatal disorders**" is understood to mean the fact that a new-born presents no sign

of prematurity, no delayed intrauterine growth, nor foetal distress. Prematurity is determined by the number of weeks of amenorrhea. Delayed intrauterine growth is determined by the birth weight, the birth size and the cranial perimeter. Foetal distress is determined by the arterial pH of the cord blood and the Apgar score defined by A = appearance (skin colour), P = heart rate, G = grimace (reflexes to stimuli), A = activity (muscle tone), R = respiration (breathing efforts). The thresholds of these parameters for determining whether a new-born has no neonatal disorders are well known to a person skilled in the art.

"**Full term childbirth**" is understood to mean childbirth resulting in the birth of a new-born having a birth weight of more than 3.2 kg, after at least 39.5 weeks of amenorrhea and the weight of the placenta at the time of childbirth being greater than 550 grams.

"**Directed labour childbirth**" is understood to mean childbirth assisted by the administration of oxytocin or the artificial rupture of the amniotic sac.

"**Clinical grade cells**" is understood to mean cells that have not come into any contact with a substance of non-human, animal origin, such as non-human, animal serum, during the isolation, culturing, cryopreservation and thawing of the cells. Clinical grade cells are cells produced under culturing conditions that are compatible with human use and in accordance with best pharmaceutical production practices.

"**Cryopreserved stem cells**" is understood to mean stem cells that are preserved at a very low temperature, preferably between -150 °C and -196 °C, preferably in the presence of a cryoprotective solution containing dimethyl sulfoxide (DMSO).

"**Mesenchymal stem cells**" is understood to mean multipotent stem cells, of mesodermal origin, found in various tissues of the adult organism, such as bone marrow, adipose tissue, as well as the umbilical cord. These stromal cells are capable of self-renewal and differentiation into cells of the osteoblastic, chondrocytic and adipocyte lineages at a minimum, including, without limitation, cells of the bone, of the cartilage, of the adipose tissue and of the medullary stroma, of smooth muscle, ligaments and tendons.

"**Thawed human mesenchymal stem cells**" is understood to mean mesenchymal stem cells that have been isolated from human tissues and have undergone the process of thawing the cryopreserved stem cells.

"**Fresh human mesenchymal stem cells**" is understood to mean mesenchymal stem cells that have been isolated from human tissues and have not undergone any freezing-thawing processes.

"**Wharton's jelly**" is understood to mean the connective tissue of extra-embryonic mesoblast origin, enveloping the two arteries and the umbilical vein and thus protecting the umbilical cord.

"**Sepsis**" is understood to mean a severe general infection of the organism by pathogenic germs, in particular bacteria. Sepsis is defined as a secondary organ dysfunction with an inappropriate response of the organism to an infection. Depending on the severity, the septic state can be classified into two orders: *stricto sensu* sepsis, and septic shock ("The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3)", JAMA. 2016; 315(8): 801-810). Sepsis, according to its strictest definition, clinically associates an infection with a SOFA score (Sequential Organ Failure Assessment Score) of ≥ 2 or an increase in the SOFA score that is greater than or equal to 2 points if an organ dysfunction existed prior to infection. Septic shock, for its part, associates sepsis with the need to use vasopressors with a Qsp/MAP [Mean Arterial Pressure] of ≥ 65 mmHg and/or a lactatemia of > 2 mmol/l (or 18 mg/dL), despite adequate vascular filling.

DETAILED DESCRIPTION

The subject matter of the present invention is mesenchymal stem cells (MSCs) for their use in the treatment of sepsis, in particular of septic shock.

In one embodiment, the MSCs are animal stem cells, preferably mammals, preferably human. According to the present invention, the MSCs are human stem cells.

In one embodiment, the MSCs are derived from Wharton's jelly, bone marrow and/or adipose tissue. According to the present invention, the MSCs are derived from Wharton's jelly.

According to the present invention, the MSCs are thawed, *i.e.*, they have undergone a freezing-thawing process.

More specifically, the subject matter of the present invention is therefore thawed human mesenchymal stem cells derived from Wharton's jelly (WJ-MSCs) for their use in the treatment of sepsis, in particular of septic shock.

According to the present invention, the thawed WJ-MSCs have phenotypic characteristics that differ from fresh WJ-MSCs.

In one embodiment, the thawed WJ-MSCs are characterized in that:

– at least 60 % of the cells express the following antigens: CD90, CD73, CD105, CD44; and

– at least 80 % of the cells do not express any of the following markers: CD34, CD11b, CD19, CD45, HLA-DR; and

– at least 10 % of the cells express the marker CD106.

In one embodiment, the thawed WJ-MSCs are characterized in that:

– at least 60 % of the cells express the following antigens: CD90, CD73, CD105, CD44; and

– at least 80 % of the cells do not express any of the following markers: CD34, CD11b, CD19, CD45, CD144, HLA-DR; and

– at least 10 % of the cells express the marker CD106.

In one embodiment, the thawed WJ-MSCs are characterized in that the expression level of CD44 is at least 20 %, at least 25 %, at least 30 %, at least 35 %, at least 40 %, at least 45 %, at least 50 % or more lower than the expression level of CD44 in fresh WJ-MSCs.

"**CD44**" is understood to mean the hyaluronan receptor, an example of which is the human CD44 protein, the UniProtKB accession number of which is P16070.

In one embodiment, the thawed WJ-MSCs are characterized in that the expression level of at least one marker selected from among CD90, CD73, CD105, CD29, CD146, CD166, HLA-ABC is at least 10 %, at least 15 %, at least 20 %, at least 25 %, at least 30 %, at least 35 %, at least 40 %, at least 45 %, at least 50 % or more lower than the expression level of the same marker in fresh WJ-MSCs.

In one embodiment, the thawed WJ-MSCs are characterized in that the expression level of the CD90 marker is at least 10 %, at least 15 %, at least 20 %, at least 25 %, at least 30 %, at least 35 %, at least 40 %, at least 45 %, at least 50 % or more lower than the expression level of the CD90 marker in fresh WJ-MSCs.

"**CD90**" is understood to mean the Thy-1 membrane glycoprotein, an example of which is human CD90 protein, the UniProtKB accession number of which is P04216.

In one embodiment, the thawed WJ-MSCs are characterized in that the expression level of the CD73 marker is at least 10 %, at least 15 %, at least 20 %, at least 25 %, at least 30 %, at least 35 %, at least 40 %, at least 45 %, at least 50 % or more lower than the expression level of the CD73 marker in fresh WJ-MSCs.

"**CD73**" is understood to mean the ecto-5'-nucleotidase (or NT5E) enzyme, an example of which is the human CD73 protein, the UniProtKB accession number of which is P21589.

In one embodiment, the thawed WJ-MSCs are characterized in that the expression level of the CD105 marker is at least 10 %, at least 15 %, at least 20 %, at least 25 %, at least 30 %, at least 35 %, at least 40 %, at least 45 %, at least 50 % or more lower than the expression level of the CD105 marker in fresh WJ-MSCs.

"**CD105**" is understood to mean the endoglin membrane glycoprotein, an example of which is human CD105 protein, the UniProtKB accession number of which is P17813.

In one embodiment, the thawed WJ-MSCs are characterized in that the expression level of the CD29 marker is at least 5 %, at least 10 %, at least 15 %, at least 20 %, at least 25 %, at least 30 %, at least 35 %, at least 40 %, at least 45 %, at least 50 % or more lower than the expression level of the CD29 marker in fresh WJ-MSCs.

"**CD29**" is understood to mean the integrin β-1 protein, an example of which is human CD29 protein, the UniProtKB accession number of which is P05556.

In one embodiment, the thawed WJ-MSCs are characterized in that the expression level of the CD146 marker is at least 10 %, at least 15 %, at least 20 %, at least 25 %, at least 30 %, at least 35 %, at least 40 %, at least 45 %, at least 50 % or more lower than the expression level of the CD146 marker in fresh WJ-MSCs.

"**CD146**" is understood to mean the Melanoma Cell Adhesion Molecule (MCAM), an example of which is human CD146 protein, the UniProtKB accession number of which is P43121.

In one embodiment, the thawed WJ-MSCs are characterized in that the expression level of the CD166 marker is at least 10 %, at least 15 %, at least 20 %, at least 25 %, at least 30 %, at least 35

%, at least 40 %, at least 45 %, at least 50 % or more lower than the expression level of the CD166 marker in fresh WJ-MSCs.

"**CD166**" is understood to mean the Activated Leukocyte Cell Adhesion Molecule (ALCAM), an example of which is human CD166 protein, the UniProtKB accession number of which is Q13740.

In one embodiment, the thawed WJ-MSCs are characterized in that the expression level of the HLA-ABC marker is at least 5 %, at least 10 %, at least 15 %, at least 20 %, at least 25 %, at least 30 %, at least 35 %, at least 40 %, at least 45 %, at least 50 % or more lower than the expression level of the HLA-ABC marker in fresh WJ-MSCs.

"**HLA-ABC**" is understood to mean the surface receptors of the class I major histocompatibility complex, an example of which is human HLA-ABC, the UniProtKB accession number of which is O19689.

In one embodiment, the thawed WJ-MSCs are characterized in that the expression level of at least one marker selected from among CD13 and Sox-2 is at least 5 %, at least 10 %, at least 15 %, at least 20 %, at least 25 %, at least 30 %, at least 35 %, at least 40 %, at least 45 %, at least 50 % or more higher than the expression level of the same marker in fresh WJ-MSCs.

In one embodiment, the thawed WJ-MSCs are characterized in that the expression level of the CD13 marker is at least 5 %, at least 10 %, at least 15 %, at least 20 %, at least 25 %, at least 30 %, at least 35 %, at least 40 %, at least 45 %, at least 50 % or more higher than the expression level of the CD13 marker in fresh WJ-MSCs.

"**CD13**" is understood to mean the membrane alanyl aminopeptidase, an example of which is human CD13 protein, the UniProtKB accession number of which is P15144.

In one embodiment, the thawed WJ-MSCs are characterized in that the expression level of the Sox-2 marker is at least 5 %, at least 10 %, at least 15 %, at least 20 %, at least 25 %, at least 30 %, at least 35 %, at least 40 %, at least 45 %, at least 50 % or more higher than the expression level of the Sox-2 marker in fresh WJ-MSCs.

"**Sox-2**" is understood to mean the SRY-box transcription factor 2, an example of which is human Sox-2 protein, the UniProtKB accession number of which is P48431.

In one embodiment, the WJ-MSCs thawed after at least one sub-culture passage are characterized in that the expression level of at least one marker selected from CD44 and SSEA-4 is at least 5 %, at least 10 %, at least 15 %, at least 20 %, at least 25 %, at least 30 %, at least 35 %, at least 40 %, at least 45 %, at least 50 % or more lower than the expression level of the same marker in fresh WJ-MSCs.

In one embodiment, the WJ-MSCs thawed after at least one sub-culture passage are characterized in that the expression level of the CD44 marker is at least 5 %, at least 10 %, at least 15 %, at least 20 %, at least 25 %, at least 30 %, at least 35 %, at least 40 %, at least 45 %, at least 50 % or more lower than the expression level of the CD44 marker in fresh WJ-MSCs.

In one embodiment, the WJ-MSCS thawed after at least one sub-culture passage are characterized in that the expression level of the SSEA-4 marker is at least 5 %, at least 10 %, at least 15 %, at least 20 %, at least 25 %, at least 30 %, at least 35 %, at least 40 %, at least 45 %, at least 50 % or more lower than the expression level of the SSEA4 marker in fresh WJ-MSCs.

"**SSEA-4**" is understood to mean the membrane ganglioside formed by a glycosphingolipid comprising a terminal sialic acid residue (Stage-Specific Embryonic Antigen 4).

In one embodiment, the WJ-MSCs thawed after at least one sub-culture passage are characterized in that the expression level of at least one marker selected from among CD90, CD166 and HLA-ABC is at least 5 %, at least 10 %, at least 15 %, at least 20 %, at least 25 %, at least 30 %, at least 35 %, at least 40 %, at least 45 %, at least 50 % or more higher than the expression level of the same marker in fresh WJ-MSCs.

In one embodiment, the WJ-MSCs thawed after at least one sub-culture passage are characterized in that the expression level of the CD90 marker is at least 5 %, at least 10 %, at least 15 %, at least 20 %, at least 25 %, at least 30 %, at least 35 %, at least 40 %, at least 45 %, at least 50 % or more higher than the expression level of the CD90 marker in fresh WJ-MSCs.

In one embodiment, the WJ-MSCs thawed after at least one sub-culture passage are characterized in that the expression level of the CD166 marker is at least 5 %, at least 10 %, at least 15 %, at least 20 %, at least 25 %, at least 30 %, at least 35 %, at least 40 %, at least 45 %, at least 50 % or more higher than the expression level of the CD166 marker in fresh WJ-MSCs.

In one embodiment, the WJ-MSCs thawed after at least one sub-culture passage are characterized in that the expression level of the HLA-ABC marker is at least 5 %, at least 10 %, at least 15 %, at least 20 %, at least 25 %, at least 30 %, at least 35 %, at least 40 %, at least 45 %, at least 50 % or more higher than the expression level of the HLA-ABC marker in fresh WJ-MSCs.

In one embodiment, the expression level of the markers is measured using methods that are well known to a person skilled in the art. In one embodiment, the expression level of the markers is measured by flow cytometry. In one embodiment, the expression level of the markers is measured by Mean Fluorescence Intensity (MFI).

In one embodiment, the thawed WJ-MSCs are characterized by a differential protein expression relative to the fresh WJ-MSCs.

In one embodiment, the thawed WJ-MSCs express at least one protein selected from among the group comprising or consisting of ACTB (cytoplasmic actin 1), ANXA1 (annexin A1), CAPZB (F-actin-capping protein sub-unit beta), LASP1 (LIM and SH3 domain protein 1), PRDX2 (peroxiredoxin-2), PRDX3 (mitochondrial thioredoxin-dependent peroxide reductase), PSA3 (proteasome sub-unit alpha type-3), RS12 (40S ribosomal protein S12) and SYWC (cytoplasmic tryptophane-tRNA ligase).

In one embodiment, the fresh WJ-MSCs do not express or do not substantially express at least one protein selected from among the group comprising or consisting of ACTB (cytoplasmic actin 1), ANXA1 (annexin A1), CAPZB (F-actin-capping protein sub-unit beta), LASP1 (LIM and SH3 domain protein 1), PRDX2 (peroxiredoxin-2), PRDX3 (mitochondrial thioredoxin-dependent peroxide reductase), PSA3 (proteasome sub-unit alpha type-3), RS12 (40S ribosomal protein S12) and SYWC (cytoplasmic tryptophane-tRNA ligase).

In one embodiment, the thawed WJ-MSCs express at least 1.1 times, 1.2 times, 1.3 times, 1.4 times, 1.5 times, 1.6 times, 1.7 times, 1.8 times, 1.9 times, 2 times, 2.5 times, 3.0 times, 3.5 times, 4.0 times, 4.5 times, 5.0 times, 7.5 times, 10.0 times, 15.0 times, 20.0 times, 30.0 times, 40.0 times, 50.0 times more of at least one protein selected from among the group comprising or consisting of ACTB (cytoplasmic actin 1), ANXA1 (annexin A1), CAPZB (F-actin-capping protein sub-unit beta), LASP1 (LIM and SH3 domain protein 1), PRDX2 (peroxiredoxin-2), PRDX3 (mitochondrial thioredoxin-dependent peroxide reductase), PSA3 (proteasome sub-unit alpha

type-3), RS12 (40S ribosomal protein S12) and SYWC (cytoplasmic tryptophan-tRNA ligase) than the fresh WJ-MSCs.

"**ACTB**" is understood to mean the cytoplasmic actin 1 protein, an example of which is human ACTB, the UniProtKB accession number of which is P60709.

"**ANXA1**" is understood to mean the annexin 1 protein (annexin A1), an example of which is human ANXA1, the UniProtKB accession number of which is P04083.

"**CAPZB**" is understood to mean the F-actin-capping protein sub-unit beta, an example of which is human CAPZB, the UniProtKB accession number of which is P47756.

"**LASP1**" is understood to mean the LIM and SH3 domain protein 1, an example of which is human LASP1, the UniProtKB accession number of which is Q14847.

"**PRDX2**" is understood to mean peroxiredoxin-2, an example of which is human PRDX2, the UniProtKB accession number of which is P32119.

"**PRDX3**" is understood to mean peroxiredoxin 3 (*mitochondrial thioredoxin-dependant peroxide reductase*), an example of which is human PRDX3, the UniProtKB accession number of which is P30048.

"**PSA3**" is understood to mean the proteasome sub-unit alpha type-3, an example of which is human PSA3, the UniProtKB accession number of which is P25788.

"**RS12**" is understood to mean the 40S ribosomal sub-unit protein S12, an example of which is human RS12, the UniProtKB accession number of which is P25398.

"**SYWC**" is understood to mean cytoplasmic tryptophane-tRNA ligase, an example of which is human SYWC, the UniProtKB accession number of which is P23381.

In one embodiment, the thawed WJ-MSCs do not express or do not substantially express at least one protein selected from among the group comprising or consisting of ACTS (skeletal muscle alpha actin), AL1B1 (mitochondrial aldehyde dehydrogenase X), ANX10 (annexin A10), GBB1 (guanine nucleotide-binding protein G(I)/G(S)/G(T) sub-unit beta-1), GBB2 (guanine nucleotide-

binding protein G(I)/G(S)/G(T) sub-unit beta-2), GPRIN1 (G protein-regulated inducer of neurite outgrowth 1), DTNA (dystrobrevin alpha), MIPO1 (mirror-image polydactyly gene 1 protein), PSB3 (proteasome sub-unit beta type-3) and PSDE (26S proteasome non-ATPase regulatory sub-unit 14).

In one embodiment, the fresh WJ-MSCs express at least one protein selected from among the group comprising or consisting of ACTS (skeletal muscle alpha actin), AL1B1 (mitochondrial aldehyde dehydrogenase X), ANX10 (annexin A10), GBB1 (guanine nucleotide-binding protein G(I)/G(S)/G(T) sub-unit beta-1), GBB2 (guanine nucleotide-binding protein G(I)/G(S)/G(T) sub-unit beta-2), GPRIN1 (G protein-regulated inducer of neurite outgrowth 1), DTNA (dystrobrevin alpha), MIPO1 (mirror-image polydactyly gene 1 protein), PSB3 (proteasome sub-unit beta type-3) and PSDE (26S proteasome non-ATPase regulatory sub-unit 14).

In one embodiment, the thawed WJ-MSCs express at least 1.1 times, 1.2 times, 1.3 times, 1.4 times, 1.5 times, 1.6 times, 1.7 times, 1.8 times, 1.9 times, 2 times, 2.5 times, 3.0 times, 3.5 times, 4.0 times, 4.5 times, 5.0 times, 7.5 times, 10.0 times, 15.0 times, 20.0 times, 30.0 times, 40.0 times, 50.0 times less of at least one protein selected from among the group comprising or consisting of ACTS (skeletal muscle alpha actin), AL1B1 (mitochondrial aldehyde dehydrogenase X), ANX10 (annexin A10), GBB1 (guanine nucleotide-binding protein G(I)/G(S)/G(T) sub-unit beta-1), GBB2 (guanine nucleotide-binding protein G(I)/G(S)/G(T) sub-unit beta -2), GPRIN1 (G protein-regulated inducer of neurite outgrowth 1), DTNA (dystrobrevin alpha), MIPO1 (mirror-image polyactyly gene 1 protein), PSB3 (proteasome sub-unit beta type-3) and PSDE (26S proteasome non-ATPase regulatory sub-unit 14) than the fresh WJ-MSCs.

"**ACTS**" is understood to mean the skeletal muscle alpha actin protein, an example of which is human ACTS, the UniProtKB accession number of which is P68133.

"**AL1B1**" is understood to mean mitochondrial aldehyde dehydrogenase X, an example of which is human AL1B1, the UniProtKB accession number of which is P30837.

"**ANX10**" is understood to mean annexin A10 protein, an example of which is human ANX10, the UniProtKB accession number of which is Q9UJ72.

"**GBB1**" is understood to mean the guanine nucleotide-binding protein G(I)/G(S)/G(T) sub-unit beta-1, an example of which is human GBB1, the UniProtKB accession number of which is

P62873.

"**GBB2**" is understood to mean the guanine nucleotide-binding protein G(I)/G(S)/G(T) sub-unit beta-2, an example of which is human GBB2, the UniProtKB accession number of which is P62879.

"**GPRIN1**" is understood to mean the G protein-regulated inducer of neurite outgrowth 1, an example of which is human GPRIN1, the UniProtKB accession number of which is Q7Z2K8.

"**DTNA**" is understood to mean dystrobrevin alpha, an example of which is human DTNA, the UniProtKB accession number of which is Q9Y4J8.

"**MIPO1**" is understood to mean the mirror-image polydactyly gene 1 protein, an example of which is human MIPO1, the UniProtKB accession number of which is Q8TD10.

"**PSB3**" is understood to mean the proteasome sub-unit beta type-3, an example of which is human PSB3, the UniProtKB accession number of which is P49720.

"**PSDE**" is understood to mean the 26S proteasome non-ATPase regulatory sub-unit 14, an example of which is the human PSDE, the UniProtKB accession number of which is O00487.

In one embodiment, the WJ-MSCs thawed after at least 1 passage in a sub-culture express at least 1.1 times, 1.2 times, 1.3 times, 1.4 times, 1.5 times, 1.6 times, 1.7 times, 1.8 times, 1.9 times, 2 times, 2.5 times, 3.0 times, 3.5 times, 4.0 times, 4.5 times, 5.0 times, 7.5 times, 10.0 times, 15.0 times, 20.0 times, 30.0 times, 40.0 times, 50. 0 times more of at least one protein selected from among the group comprising or consisting of AMPM2 (methionine aminopeptidase 2), DJB11 (dnaJ homolog subfamily B member 11), K1C18 (keratin, type I cytoskeletal 18), K1C19 (keratin, type I cytoskeletal 19), K2C8 (keratin type II cytoskeletal 8), LYSC (lysozyme C), PDIA3 (protein disulfide-isomerase A3), TCTP (translationally-controlled tumour protein) and TGM2 (protein-glutamine gamma-glutamyltransferase 2) than the fresh WJ-MSCs.

"**AMPM2**" is understood to mean methionine aminopeptidase 2, an example of which is human AMPM2, the UniProtKB accession number of which is P50579.

"**DJB11**" is understood to mean dnaJ homolog sub-family B member 11 of HSP40, an example of

which is human DJB11, the UniProtKB accession number of which is Q9UBS4.

"**K1C18**" is understood to mean keratin, type I cytoskeletal 18 acid, an example of which is human K1C18, the UniProtKB accession number of which is P05783.

"**K1C19**" is understood to mean keratin, type I cytoskeletal 19 acid, an example of which is human K1C19, the UniProtKB accession number of which is P08727.

"**K2C8**" is understood to mean keratin type II cytoskeletal 8 basic, an example of which is human K2C8, the UniProtKB accession number of which is P05787.

"**LYSC**" is understood to mean lysozyme C, an example of which is human LYSC, the UniProtKB accession number of which is P61626.

"**PDIA3**" is understood to mean the protein disulfide-isomerase A3 enzyme, an example of which is human PDIA3, the UniProtKB accession number of which is P30101.

"**TCTP**" is understood to mean the translationally-controlled tumour protein, an example of which is human TCTP, the UniProtKB accession number of which is P13693.

"**TGM2**" is understood to mean the protein-glutamine gamma-glutamyltransferase 2 enzyme, an example of which is human TGM2, the UniProtKB accession number of which is P21980.

In one embodiment, the WJ-MSCs thawed after at least 1 passage in a sub-culture express at least 1.1 times, 1.2 times, 1.3 times, 1.4 times, 1.5 times, 1.6 times, 1.7 times, 1.8 times, 1.9 times, 2 times, 2.5 times, 3.0 times, 3.5 times, 4.0 times, 4.5 times, 5.0 times, 7.5 times, 10.0 times, 15.0 times, 20.0 times, 30.0 times, 40.0 times, 50.0 times less of at least one protein selected from among the group comprising or consisting of GSTP1 (glutathione S-transferase P), HSP7C (heat shock cognate 71 kDa protein), HSPB1 (heat shock protein beta-1), LEG1 (galectin-1), S10AB (protein S100-A11) and UBE2N (ubiquitin-conjugating enzyme E2 N) than the fresh WJ-MSCs.

"**GSTP1**" is understood to mean the glutathione S-transferase P enzyme, an example of which is human GSTP1, the UniProtKB accession number of which is P09211.

"**HSP7C**" is understood to mean the heat shock cognate 71 kDa protein, an example of which is

human HSP7C, the UniProtKB accession number of which is P11142.

"**HSPB1**" is understood to mean the heat shock protein beta-1, an example of which is human HSPB1, the UniProtKB accession number of which is P04792.

"**LEG1**" is understood to mean galectin-1, an example of which is human LEG1, the UniProtKB accession number of which is P09382.

"**S10AB**" is understood to mean the protein S100-A11, an example of which is human S10AB, the UniProtKB accession number of which is P31949.

"**UBE2N**" is understood to mean the ubiquitin-conjugating enzyme E2 N, an example of which is human UBE2N, the UniProtKB accession number of which is P61088.

In one embodiment, the protein expression is measured using methods that are well known to a person skilled in the art. In one embodiment, the protein expression is measured using mass spectrometry.

In one embodiment, the WJ-MSCs thawed under *in vitro* conditions and/or non-inflammatory conditions secrete at least one growth factor selected from among BMP-7, IGFBP-1, insulin, FGF-7, NT-4 and VEGF-D.

In one embodiment, the WJ-MSCs thawed under *in vitro* conditions and/or non-inflammatory conditions secrete at least 1.1 times, 1.2 times, 1.3 times, 1.4 times, 1.5 times, 1.6 times, 1.7 times, 1.8 times, 1.9 times, 2 times, 2.5 times, 3.0 times, 3.5 times, 4.0 times, 4.5 times, 5.0 times, 7.5 times, 10.0 times, 15.0 times, 20.0 times, 30.0 times, 40.0 times, 50. 0 times more of at least one growth factor selected from among BMP-7, IGFBP-1, insulin, FGF-7, NT-4 and VEGF-D than the fresh WJ-MSCs, under *in vitro* conditions and/or non-inflammatory conditions.

In one embodiment, the WJ-MSCs thawed under *in vitro* conditions and/or non-inflammatory conditions secrete at least 50 pg/mL, at least 100 pg/mL, at least 150 pg/mL, at least 200 pg/mL, at least 250 pg/mL, at least 300 pg/mL, at least 350 pg/mL, at least 400 pg/mL, at least 450 pg/mL, at least 500 pg/mL or more of BMP-7.

In one embodiment, the WJ-MSCs thawed under *in vitro* conditions and/or non-inflammatory

conditions secrete at least 20 pg/mL, at least 25 pg/mL, at least 30 pg/mL, at least 35 pg/mL, at least 40 pg/mL, at least 45 pg/mL, at least 50 pg/mL, at least 55 pg/mL, at least 60 pg/mL, at least 65 pg/mL or more of IGFBP-1.

In one embodiment, the WJ-MSCs thawed under *in vitro* conditions and/or non-inflammatory conditions secrete at least 50 pg/mL, at least 75 pg/mL, at least 100 pg/mL, at least 125 pg/mL, at least 150 pg/mL, at least 175 pg/mL, at least 200 pg/mL, at least 225 pg/mL, at least 250 pg/mL, at least 275 pg/mL or more of insulin.

In one embodiment, the WJ-MSCs thawed under *in vitro* conditions and/or non-inflammatory conditions secrete at least 10 pg/mL, at least 15 pg/mL, at least 20 pg/mL, at least 25 pg/mL, at least 30 pg/mL, at least 35 pg/mL, at least 40 pg/mL, at least 45 pg/mL, at least 50 pg/mL, at least 55 pg/mL, at least 60 pg/mL, at least 65 pg/mL or more of FGF-7.

In one embodiment, the WJ-MSCs thawed under *in vitro* conditions and/or non-inflammatory conditions secrete at least 45 pg/mL, at least 50 pg/mL, at least 55 pg/mL, at least 60 pg/mL, at least 65 pg/mL, at least 70 pg/mL, at least 75 pg/mL, at least 80 pg/mL, at least 85 pg/mL, at least 90 pg/mL or more of NT-4.

In one embodiment, the WJ-MSCs thawed under *in vitro* conditions and/or non-inflammatory conditions secrete at least 5 pg/mL, at least 10 pg/mL, at least 15 pg/mL, at least 20 pg/mL, at least 25 pg/mL, at least 30 pg/mL, at least 35 pg/mL, at least 40 pg/mL, at least 45 pg/mL, at least 50 pg/mL or more of VEGF-D.

In one embodiment, the WJ-MSCs thawed under *in vivo* conditions and/or inflammatory conditions secrete at least one growth factor selected from among BMP-7 and TGFβ3.

In one embodiment, the WJ-MSCs thawed under *in vivo* conditions and/or inflammatory conditions secrete at least 1.1 times, 1.2 times, 1.3 times, 1.4 times, 1.5 times, 1.6 times, 1.7 times, 1.8 times, 1.9 times, 2 times, 2.5 times, 3.0 times, 3.5 times, 4.0 times, 4.5 times, 5.0 times, 7.5 times, 10.0 times, 15.0 times, 20.0 times, 30.0 times, 40.0 times, 50.0 times more of at least one growth factor selected from among BMP-7 and TGFβ3 than fresh WJ-MSCs, under *in vivo* conditions and/or inflammatory conditions.

In one embodiment, the WJ-MSCs thawed under *in vivo* conditions and/or inflammatory

conditions secrete at least 200 pg/mL, at least 250 pg/mL, at least 300 pg/mL, at least 350 pg/mL, at least 400 pg/mL, at least 450 pg/mL, at least 500 pg/mL, at least 550 pg/mL, at least 600 pg/mL, at least 650 pg/mL, at least 700 pg/mL, at least 750 pg/mL or more of BMP-7.

In one embodiment, the WJ-MSCs thawed under *in vivo* conditions and/or inflammatory conditions secrete at least 20 pg/mL, at least 25 pg/mL, at least 30 pg/mL, at least 35 pg/mL, at least 40 pg/mL, at least 45 pg/mL, at least 50 pg/mL, at least 55 pg/mL, at least 60 pg/mL, at least 65 pg/mL or more of TGFβ3.

In one embodiment, the WJ-MSCs thawed under *in vivo* conditions and/or inflammatory conditions do not secrete or do not substantially secrete IGFBP-1.

In one embodiment, the WJ-MSCs thawed under *in vivo* conditions and/or inflammatory conditions secrete at least 1.1 times, 1.2 times, 1.3 times, 1.4 times, 1.5 times, 1.6 times, 1.7 times, 1.8 times, 1.9 times, 2 times, 2.5 times, 3.0 times, 3.5 times, 4.0 times, 4.5 times, 5.0 times, 7.5 times, 10.0 times, 15.0 times, 20.0 times, 30.0 times, 40.0 times, 50.0 times less of IGFBP-1 than fresh WJ-MSCs under *in vivo* conditions and/or inflammatory conditions.

In one embodiment, the WJ-MSCs thawed under *in vivo* conditions and/or inflammatory conditions secrete less than 20 pg/mL, at least 15 pg/mL, at least 10 pg/mL, at least 5 pg/mL, at least 4 pg/mL, at least 3 pg/mL, at least 2 pg/mL, at least 1 pg/mL or less of IGFBP-1.

"**BMP-7**" is understood to mean Bone Morphogenetic Protein 7. An example of BMP-7 corresponds to the human BMP-7 protein, the UniProtKB accession number of which is P18075.

"**IGFBP-1**" is understood to mean the Insulin-like Growth Factor-Binding Protein 1. An example of IGFBP-1 corresponds to the human IGFBP-1 protein, the UniProtKB accession number of which is P08833.

"**Insulin**" is understood to mean the protein hormone that promotes the absorption of blood glucose. An example of insulin corresponds to human insulin, the UniProtKB accession number of which is P01308.

"**FGF-7**" is understood to mean Fibroblast Growth Factor 7. An example of FGF-7 corresponds to the human FGF-7 protein, the UniProtKB accession number of which is P21781.

"**NT-4**" is understood to mean neurotrophin 4. An example of NT-4 corresponds to human NT-4 protein, the UniProtKB accession number of which is P34130.

"**VEGF-D**" is understood to mean Vascular Endothelial Growth Factor D. An example of VEGF-D corresponds to the human VEGF-D protein, the UniProtKB accession number of which is O43915.

"**TGFβ3**" is understood to mean Transforming Growth Factor β3. An example of TGFβ3 corresponds to the human TGFβ3 protein, the UniProtKB accession number of which is P10600.

"***In vitro* conditions**" is understood to mean conditions in which the thawed WJ-MSCs are outside a living organism, preferably outside the patient.

"***In vivo* conditions**" is understood to mean conditions in which the thawed WJ-MSCs are inside a living organism, preferably inside the patient, *i.e.*, after they are administered to the patient.

"**Non-inflammatory conditions**" is understood to mean conditions in which the thawed WJ-MSCs are not stimulated, *in vitro* or *in vivo*, by inflammatory cytokines, including TNF-α and IFN-γ. In particular, non-inflammatory conditions can be defined as *in vivo* conditions, preferably inside the patient, said patient being healthy or substantially healthy, *i.e.*, said patient is not affected by sepsis.

"**Inflammatory conditions**" is understood to mean conditions in which the thawed WJ-MSCs are stimulated, *in vitro* or *in vivo*, by inflammatory cytokines, including TNF-α and IFN-γ. In particular, inflammatory conditions can be defined as *in vivo* conditions, preferably inside the patient, said patient being affected by sepsis.

In one embodiment, the WJ-MSCs are preserved at a very low temperature. In one embodiment, the WJ-MSCs are preserved at approximately -20 °C, preferably at approximately -80 °C, preferably between -150 °C and -196 °C. In one embodiment, the WJ-MSCs are preserved in the presence of a cryoprotective solution. The cryoprotective solutions of cells are well known to a person skilled in the art. Such solutions comprise, *e.g.*, In one embodiment, the cryoprotective solution comprises at least one cryoprotective agent selected from among dimethyl sulfoxide (DMSO), glycerol, ethylene glycol, propylene glycol, formamide, butene diol and a mixture

thereof.

In one embodiment, the WJ-MSCs are thawed in accordance with a conventional thawing protocol. By way of an example, the WJ-MSCs are thawed in a water bath at 37 °C and then washed with a washing solution comprising NaCl, albumin and citrate dextrose formula A (ACD).

A particular embodiment of the invention relates to thawed WJ-MSCs as described above, for their use in the treatment of septic shock, the most severe form of sepsis, which is defined by the onset or persistence of arterial hypotension and/or signs of peripheral hypoperfusion despite adequate vascular filling.

In an advantageous embodiment, the WJ-MSCs used in the invention are directly derived from thawing, without reculturing after thawing.

Unlike the previous studies, in which the analysed cells are fresh cells, which have been neither frozen nor thawed, and were administered in the early stages in animals in a septic shock model, the results of the present invention for the first time provide evidence of the efficacy of WJ-MSCs under conditions compatible with clinical conditions, namely the administration of thawed WJ-MSCs in the late stages of the evolution of the sepsis.

The present invention shows that the thawed WJ-MSCs preserve the immunomodulatory and antibacterial properties at a level that is comparable to those of MSCs that are isolated from thawed bone marrow (BM-MSC) and that administering WJ-MSCs even allows the survival of mice suffering from septic shock to be increased compared to the survival of mice treated with BM-MSCs.

These results show that thawed WJ-MSCs are able to be immediately used under clinical conditions for the treatment of septic shock.

In one embodiment, the WJ-MSCs used in the invention are washed after thawing. In one embodiment, the WJ-MSCs used in the invention are resuspended after thawing. In one embodiment, the WJ-MSCs used in the invention are resuspended after thawing in a solution of hydroxyethyl starch. In one embodiment, the WJ-MSCs used in the invention are resuspended after thawing in an albumin solution. In one embodiment, the albumin solution is a 4 % albumin solution. In one embodiment, the albumin solution further comprises NaCl and/or citrate dextrose

formula A (ACD).

In the present disclosure, the inventors have also observed, for the first time, a significant correlation between, on the one hand, obstetric factors and, on the other hand, the proliferation of WJ-MSCs and the availability of the criteria for selecting umbilical cords in order to obtain WJ-MSCs with a better proliferation capacity.

In one embodiment, the WJ-MSCs used in the present invention are derived from a human umbilical cord tissue that meets these criteria.

It has been noted that these criteria improve cell proliferation by decreasing the doubling time of the number of cells, in particular during passage P1. These criteria also allow a greater number of cells to be obtained with a short doubling time.

In one embodiment, the WJ-MSCs used in the present invention are derived from a human umbilical cord tissue originating from a mother meeting at least one of the following criteria: having received an administration of oxytocin during childbirth, having given birth by directed labour, having given birth at full term, not having presented pre-eclampsia during the pregnancy, whose child has not presented neonatal disorders and has been subject to an intake of tobacco smoke during the pregnancy.

The inventors have noted that these obstetric factors have a positive impact on the cell proliferation of WJ-MSCs and allow WJ-MSCs to be selected with better proliferation properties.

In one embodiment, the WJ-MSCs used in the present invention are clinical grade cells.

Consequently, any culture medium and reagent used are devoid of a substance of non-human, animal origin. In one embodiment, any culture medium and reagent used are devoid of a serum of non-human, animal origin. In one embodiment, any culture medium and reagent used are devoid of a serum of any origin, even of human origin.

Advantageously, the culture medium used for the adhesion of cells contains human platelet lysate.

The subject matter of the present invention is also a composition comprising thawed WJ-MSCs as described above, for use in the treatment of sepsis.

The subject matter of the present invention is also a pharmaceutical composition comprising, as active ingredients, clinical grade thawed WJ-MSCs as described above, for use in the treatment of sepsis.

Said composition further comprises a pharmaceutically acceptable excipient.

Within the scope of the present disclosure, a pharmaceutically acceptable excipient is an excipient devoid of a substance of non-human, animal origin and suitable for use in contact with the cells of human individuals without toxicity, irritation, or an induced allergic response. A person skilled in the art will know how to select a pharmaceutically acceptable excipient according to the galenic formulation of the composition and its mode of administration.

By way of an example, said excipient is 4 % albumin or a solution of hydroxyethyl starch (HEA) 130/0.42.

Said pharmaceutical composition can be in the form of an infusion product and packaged in an infusion bag.

The present disclosure also describes a drug that comprises thawed WJ-MSCs as described above, for use in the treatment of sepsis.

In one embodiment, the WJ-MSCs used in the invention can be administered to the patient by the systemic or local route.

In one embodiment, the WJ-MSCs used in the invention can be administered to the patient by the intravenous, intravascular, intracerebral, parenteral, intraperitoneal, epidural, intra-spinal, intrastromal, intra-articular, intra-synovial, intrathecal, intraarterial, intracardiac or intramuscular route.

In one embodiment, the WJ-MSCs used in the invention can be administered to the patient by bolus injection (also referred to as rapid injection) or by continuous infusion (also referred to as slow injection).

In a preferred embodiment, the clinical grade thawed WJ-MSCs for use in the treatment of sepsis

are administered intravenously.

In one embodiment, the previously described clinical grade thawed WJ-MSCs can be administered to a patient suffering from sepsis with a dose of approximately 0.3×10^6 to 3×10^6 cells per kg of weight, in particular a dose of 1×10^6 cells per kg of weight. A person skilled in the art can adjust the dose according to the severity of the infection, the weight of the patient, the number or frequency of administration.

In one embodiment, the WJ-MSCs used in the invention can be administered to the patient at a dose ranging from 10^3 to 10^9 MSC/kg, preferably from 10^4 to 10^8 MSC/kg, preferably from 10^5 to 10^7 MSC/kg.

In one embodiment, the WJ-MSCs used in the invention can be administered to the patient at a dose of 1×10^5 MSC/kg, 2×10^5 MSC/kg, 3×10^5 MSC/kg, 4×10^5 MSC/kg, 5×10^5 MSC/kg, 6×10^5 MSC/kg, 7×10^5 MSC/kg, 8×10^5 MSC/kg, 9×10^5 MSC/kg, 1×10^6 MSC/kg, 2×10^6 MSC/kg, 3×10^6 MSC/kg, 4×10^6 MSC/kg, 5×10^6 MSC/kg, 6×10^6 MSC/kg, 7×10^6 MSC/kg, 8×10^6 MSC/kg, 9×10^6 MSC/kg, 1×10^7 MSC/kg.

In one embodiment, the WJ-MSCs used in the invention can be administered to the patient at a dose ranging from 10^3 to 10^9 MSC/kg/day, preferably from 10^4 to 10^8 MSC/kg/day, preferably from 10^5 to 10^7 MSC/kg/day.

In one embodiment, the WJ-MSCs used in the invention can be administered to the patient at a dose of 1×10^5 MSC/kg/day, 2×10^5 MSC/kg/day, 3×10^5 MSC/kg/day, 4×10^5 MSC/kg/day, 5×10^5 MSC/kg/day, 6×10^5 MSC/kg/day, 7×10^5 MSC/kg/day, 8×10^5 MSC/kg/day, 9×10^5 MSC/kg/day, 1×10^6 MSC/kg/day, 2×10^6 MSC/kg/day, 3×10^6 MSC/kg/day, 4×10^6 MSC/kg/day, 5×10^6 MSC/kg/day, 6×10^6 MSC/kg/day, 7×10^6 MSC/kg/day, 8×10^6 MSC/kg/day, 9×10^6 MSC/kg/day, 1×10^7 MSC/kg/day.

In one embodiment, the WJ-MSCs MSCs used in the invention can be administered to the patient at a dose ranging from 10^3 to 10^9 MSC/day, preferably from 10^4 to 10^8 MSC/day, preferably from 10^5 to 10^7 MSC/day.

In one embodiment, the WJ-MSCs used in the invention can be administered to the patient at a dose of 1×10^5 MSC/kg/day, 2×10^5 MSC/day, 3×10^5 MSC/day, 4×10^5 MSC/day, $5 \times$

10^5 MSC/day, 6×10^5 MSC/day, 7×10^5 MSC/day, 8×10^5 MSC/day, 9×10^5 MSC/day, 1×10^6 MSC/day, 2×10^6 MSC/day, 3×10^6 MSC/day, 4×10^6 MSC/day, 5×10^6 MSC/day, 6×10^6 MSC/day, 7×10^6 MSC/day, 8×10^6 MSC/day, 9×10^6 MSC/day, 1×10^7 MSC/day.

In one embodiment, the WJ-MSCs used in the invention can be administered in a single dose, or in multiple doses spaced apart over time.

In one embodiment, the WJ-MSCs used in the invention can be administered once daily, twice daily, three times per day or more.

In one embodiment, the WJ-MSCs used in the invention can be administered every day, every two days, every three days, every four days, every five days, every six days.

In one embodiment, the WJ-MSCs used in the invention can be administered once a week, every week, every 2 weeks, every 3 weeks.

In one embodiment, the WJ-MSCs used in the invention can be administered once a month, every month, every 2 months, every 3 months, every 4 months, every 5 months, every 6 months or more.

According to the invention, the previously described thawed WJ-MSCs can be diluted in a suitable excipient prior to use, for example, a 4 % albumin solution (4 g/100 mL) or a solution of hydroxyethyl starch (HEA) 130/0.42.

The thawed WJ-MSCs for the treatment of sepsis according to the present invention can be used alone, or before, during, or after another sepsis treatment, in particular an antibiotic treatment.

In one embodiment, the sepsis treatments are well known to a person skilled in the art, and include antibiotic therapy, antifungal therapy, vascular filling, the administration of vasopressors, and corticotherapy.

In an advantageous embodiment, the clinical grade thawed WJ-MSCs for use in the treatment of sepsis are administered to patients suffering from sepsis undergoing antibiotic treatment.

In one embodiment, the patient is being treated with at least one antibiotic. In one embodiment, the patient is being treated with at least two antibiotics. In one embodiment, the patient is being

treated with at least 3 or more antibiotics.

Examples of antibiotics for the treatment of sepsis include ampicillin, azithromycin, aztreonam, cefazolin, cefepime, clindamycin, levofloxacin, linezolid, meropenem, metronidazole, piperacillin, tazobactam, tobramycin and vancomycin.

In one embodiment, the clinical grade thawed WJ-MSCs for use in the treatment of sepsis are administered to patients suffering from sepsis undergoing antifungal treatment.

In one embodiment, the patient is being treated with at least one antifungal agent. In one embodiment, the patient is being treated with at least two antifungal agents. In one embodiment, the patient is being treated with at least 3 or more antifungal agents.

Examples of antifungal agents for the treatment of sepsis include amphotericin B deoxycholate, anidulafungin, caspofungin, fluconazole, itraconazole, micafungin and voriconazole.

In one embodiment, the clinical grade thawed WJ-MSCs for use in the treatment of sepsis are administered to patients suffering from sepsis undergoing vascular filling.

Vascular filling allows hypovolemia to be corrected, the mean arterial pressure to be kept above 65 mmHg and the clinical signs of hypoperfusion to be limited, by restoring the intravascular volume. Vascular filling is carried out by means of solutes.

Examples of solutes for vascular filling in the treatment of sepsis include colloids and crystalloids.

In one embodiment, the clinical grade thawed WJ-MSCs for use in the treatment of sepsis are administered to patients suffering from sepsis undergoing treatment with vasopressors.

In one embodiment, the patient is being treated with at least one vasopressor. In one embodiment, the patient is being treated with at least two vasopressors. In one embodiment, the patient is being treated with at least 3 or more vasopressors.

Examples of vasopressors for the treatment of sepsis include catecholamines (including noradrenaline and adrenaline) and vasopressin.

In one embodiment, the clinical grade thawed WJ-MSCs for use in the treatment of sepsis are administered in patients suffering from sepsis undergoing treatment with corticoids.

In one embodiment, the patient is being treated with at least one corticoid. In one embodiment, the patient is being treated with at least two corticoids. In one embodiment, the patient is being treated with at least 3 or more corticoids.

Examples of corticoids for the treatment of sepsis include cortisone, hydrocortisone and prednisone.

Other treatments are suitable for sepsis, and are well known to a person skilled in the art. Examples of such treatments include AB103, recombinant alkaline phosphatase recAP, CaCP29, activated drotrecogin alfa, EA-230, eritoran, esmolol, GM-CSF, IFNγ, lenercept, levosimendan, LR12, selepressin, talactoferrin alpha and recombinant human thrombomodulin.

BRIEF DESCRIPTION OF THE FIGURES

The present invention is illustrated in further detail in the figures and the examples described hereafter.

Figures 1A and 1B: these figures respectively represent the average anaerobic (figure 1A) or aerobic (figure 1B) count of CFU per milligram of spleen. The results are presented as an average ± WEEK. n = 7-12 mice per group. $*p < 0.05$ MSC versus PBS.

Figures 2A and 2B: these figures respectively represent the anaerobic (Figure 2A) or aerobic (Figure 2B) count of CFU per ml of blood. The results are presented as an average ± WEEK. n = 7-12 mice per group. $*p < 0.05$ MSC versus PBS.

Figures 3A, 3B, 3C, 3D: these figures respectively represent the kinetics of neutrophils in the lung (Figure 3A), the spleen (Figure 3B), the liver (Figure 3C), the femur (Figure 3D). The results are presented as an average ± WEEK. n = 4-6 mice per group. $*p < 0.05$ MSC versus PBS; $**p < 0.01$ MSC versus PBS; $***p < 0.001$ MSC versus PBS.

Figures 4A, 4B, 4C, 4D: these figures respectively represent the kinetics of monocytes in the lung (Figure 4A), the femur (Figure 4B), or inflammatory monocytes in the lung (Figure 4C), the femur (Figure 4D). The results are presented as an average ± WEEK. n = 4-6 mice per group. $*p < 0.05$

MSC versus PBS; ∗∗p < 0.01 MSC versus PBS; ##p < 0.01 BM-MSCs versus WJ-MSCs.

Figure 5: this figure shows the survival rate after ligation and perforation of the *cæcum* of mice treated with WJ-MSCs, BM-MSCs and of untreated mice. The results are presented in the form of Kaplan-Meier curves. n = 18-25 mice per group. ∗p < 0.05 MSC versus PBS.

Figures 6A, 6B, 6C, 6D, 6E, 6F, 6G, 6H: these figures show the impact of the WJ-MSCs treatment on sepsis in pigs compared to pigs without treatment. Figure 6A compares the venous oxygen saturation as a function of time in the group with the WJ-MSCs treatment and in the group without the treatment (∗∗p < 0.01 WJ-MSCs *versus* control group). Figure 6B compares the reduction in mean arterial pressure as a function of time in the group with the WJ-MSCs treatment and in the group without the treatment (∗∗p < 0.01 WJ-MSCs *versus* control group). Figure 6C compares the rate of noradrenaline administered as a function of time in the group with the WJ-MSCs treatment and that in the group without the treatment (∗∗∗∗p < 0.0001 WJ-MSCs *versus* control group). Figure 6D compares the plasma creatinine level as a function of time in the group with the WJ-MSCs treatment and in the group without the treatment (∗∗p < 0.01 WJ-MSCs *versus* control group). Figure 6E compares the diuresis in the group with the WJ-MSCs treatment and in the group without the treatment. Figure 6F compares the rate of lactatemia as a function of time in the group with the WJ-MSCs treatment and in the group without the treatment (∗∗p < 0.01 WJ-MSCs *versus* control group). Figure 6G compares the ratio PaO_2:FiO_2 as a function of time in the group with the WJ-MSCs treatment and in the group without the treatment (∗p < 0.5 WJ-MSCs *versus* control group). Figure 6H compares the survival of animals as a function of time in the group with the WJ-MSCs treatment and in the group without the treatment.

Figures 7A, 7B: these figures show the impact of obstetric factors on cell proliferation (Figures 7A and 7B). The doubling time is inversely proportional to the proliferation. ∗: p ≤ 0.05 in bi-variate regression, ∗∗: p ≤ 0.05 in multivariate regression.

EXAMPLES

Example 1: Production of WJ-MSCs

Isolation of WJ-MSCs

The human umbilical cords were collected from the Nancy CHRU Maternity ward after informing the donor mothers and obtaining their written consent. The cords were placed in a sampling medium and preserved at 4 °C. The sampling medium, which is placed in a sterile box (for example, Cryokits, Verreries Talançonnaises), is made up of a saline phosphate buffer (PBS,

Macopharma, BC0120020) in order to ensure a pH of close to 7, of heparin in order to prevent the residual blood from coagulating, of antibiotic (Gentamicin). The cord can be preserved in the transport medium for 24 hours at 4 °C after its removal.

Upon receipt, the umbilical cords are immersed for one hour at room temperature in an antibiotic-antifungal solution that is made up of gentamicin, amoxicillin, vancomycin and amphotericin B in a clinical grade α-MEM culture medium (Macopharma, BC0110010) with a respective final concentration of 0.5 g/L; 1g/L; 1g/L and 0.05 g/L. This bath allows the risks of microbial growth during culturing to be reduced, which would be secondary to contamination during sampling.

Once the cord is decontaminated, the vein and the outer part of the cord are washed with PBS. 10 mL of PBS is injected into the umbilical vein using a syringe in order to remove any residual blood. The cord is then pre-cut into 5 cm pieces using a sterile scalpel and then thin transverse cuts (2 to 3 mm thick) are made. These pieces are then placed in culture dishes with detachable lids (TPP, 90552). Dry adhesion of the pieces on the bottom of the dishes is implemented for 15 minutes and then the complete medium, made up of α-MEM supplemented with 5 % of platelet lysate (Macopharma, BC0190020) in the presence of gentamycin and heparin, is added. Culturing is carried out in a dedicated incubator at 37 °C and with 5 % of CO_2 and in hypoxia (5 % of O_2). After 5 days, the culture medium is changed.

After approximately 10 days of culturing, the WJ-MSCs will have migrated and adhered to the bottom of the culture dishes. The pieces are then removed using sterile tongs and the dish is washed with the PBS buffer. The cells are then cultured until confluence (> 80%) for between 15 days and 3 weeks (between D14 and D21).

Culturing of WJ-MSCs

Once the confluence is obtained, the cells are detached by the action of the recombinant trypsin GMP (TrFE, Invitrogen).

After washing, the cells are reseeded at 1×10^3 cells per cm^2 in passage 1 in the complete medium. A 2-stage culture unit with a 1,270 cm^2 surface area of the CellSTACK type (CELLSTACK2, Macopharma) is used along with a universal plug with a stack (MPC plug, Macopharma). The seeding kit (BC0400011, Macopharma) comprises the tube system allowing the various components of the medium and cells to be supplied, as well as the connector for connecting to the culture container.

The medium is changed once a week until confluence is obtained (> 80 %) (between D7 and D8 from the start of culturing). To this end, a medium replacement kit (BC0400021, Macopharma) comprising the tube system for supplying the various components of the medium, a waste pouch, and the connector for connecting to the culture container is used, as well as a universal plug with a stack. However, most of the time, the passage P1 lasts for 1 week and replacement of the medium is not required.

Once the confluence is obtained, the cells are cryopreserved in a Master Cell Bank (MCB). The Food and Drug Administration (FDA) defines an MCB as a collection of cells with a uniform composition, derived from a single tissue or cell, and stored as aliquot under defined conditions.

Final harvesting of the cells

After the confluence is obtained at the end of passage P1, the cells are harvested through the action of trypsin, as described above, and then washed. A viability check and cell count, as well as the various checks of P1, are carried out (see the table below). The cells are then frozen.

Preservation of the MSCs of the MCB

The MSCs are stored in tanks (in nitrogen vapour) at -150 °C with continuous recording of the temperature and the nitrogen level. Moreover, 5 to 10 MSC sample tubes are also frozen for carrying out the checks.

Thawing of the MCB and washing of the MSCs

The MSCs are thawed and washed using a Sepax-type technique (Biosafe) and then reseeded in passage 2 and then 3 in accordance with the same protocol as for P1.

Final harvesting of the cells

After the confluence is obtained at the end of passage P3, the cells are harvested through the action of trypsin, as described above, and then washed. A viability check and cell count, as well as the various end of production checks, are carried out (see the table below). The cells are then frozen in the Working Cell Bank (WCB). The Food and Drug Administration (FDA) defines a WCB as a collection of cells derived from one or more aliquot(s) of an MCB. The MCB cells are expanded by sub-cultures in series up to the selected passage, after which the cells are combined, concentrated, and aliquoted. One or more aliquot(s) of the WCB obtained thus can be used to produce a batch of the final product for the use thereof.

Preservation of the MSCs of the WCB

The MSCs are stored in tanks (in nitrogen vapour) at -150 °C with continuous recording of the temperature and the nitrogen level. Moreover, 5 to 10 MSC sample tubes are also frozen for carrying out the checks.

Thawing, washing of the MSCs of the WCB and routing of the MSCs

The MSCs are thawed and washed using a Sepax-type technique (Biosafe) and then returned to a 75 mL volume with 4 % albumin and preserved between 4 and 10 °C. The MSCs are packaged in a pouch (150 ml pouch), then labelled in accordance with the regulatory procedure; said pouch is placed in a secondary package (plastic pouch), which is also labelled, and then in a transport container with a temperature recorder, a validation certificate, an injection notice and a post-administration tolerance sheet. A CHRU approved carrier transports the MSCs to the delivery site.

Quality checks carried out throughout the production process

In order to ensure the sterility and the safety of the product to be administered, product checks are carried out at each stage of the culturing process. All the quality checks are listed in **Table 1**.

Table 1

Steps	**Checks carried out at each step**
Reception of the sample:	Infectious markers of the donor (Serologies / DGV)
Replacement of the medium The medium is replaced once a week	Sterility check
Trypsination: end of P0	Count Cell viability Cell phenotype Sterility check CFU-F

Trypsination: end of P1 MCB	Count Cell viability Cell phenotype Sterility check Check for the absence of endotoxin and of mycoplasmas CFU-F Check for the cell differentiation of mesodermic tissues Telomerase (hTERT) Karyotype Mixed lymphocyte culture Potency test (definition ongoing)
Trypsination: end of P2	Count Cell viability Cell phenotype Sterility check CFU-F
Final harvesting: end of P3 (pre-freezing). After obtaining the confluence at the end of P_3, the MSCs are trypsinated, washed and then frozen.	Count Cell viability Cell phenotype Sterility check Check for the absence of endotoxin and of mycoplasmas CFU-F Check for the cell differentiation of mesodermic tissues Telomerase (hTERT) Karyotype Mixed lymphocyte culture Potency test (definition ongoing)

Cell checks

Cell count

On the starting product, and during each cell harvesting step (P0 and P3), the cell count is determined using a hemacytometer following the lysis of the red blood cells.

Cell viability

On the starting product and during each cell harvesting step, the viability is determined by flow cytometry by 7 AAD marking at the end of P0, P1, P2, P3, which must be > 80 %.

Cell phenotype

The expression of the characteristic surface markers of a cell type will be determined by flow cytometry after the cells are marked with monoclonal antibodies or the corresponding isotypical checks.

At least 60 % of the cells must express the following antigens: CD90, CD73, CD105, CD44 and 80 % of the cells must not express: CD34, CD11b, CD19, CD45, HLA-DR (and/or IMF < 2 times IMF of the isotypical check). The CD106 marker must be identified on 10 % of the cells at least.

CFU-F

The clonogenic capacity will be measured by culturing fibroblast progenitors (colony forming unit fibroblast: CFU-F). The CFU-F (> 50 cells) will be counted on an inverted microscope with 10 x magnification, following attachment to methanol and Giemsa staining. In brief, 2.5 and 5 x 10^2 cells are seeded in 5 mL of medium in a 25 cm^2 flask. The medium is entirely renewed 2 times per week. Culturing is stopped on D10, it is attached and Giemsa stained. Colonies of more than 50 cells are then counted. CFU-F culturing is carried out at the end of each passage.

Immunological check

The absence of immunostimulatory capacity of the MSCs is checked. To this end, a mixed lymphocyte culture will be produced using the MSC produced and irradiated as stimulating cells and the mononuclear cells of 2 healthy control samples. The immunostimulating effect is assessed by measuring the stimulation index. These tests are carried out in the quality control sector of the department for advanced therapy medicinal products prepared on a non-routine basis (MTI-PP) of the Nancy Cell Therapy and Tissue Bank Unit (UTCT). The immunomodulation effect is assessed by adding MSCs as a third party to a conventional lymphocyte mixed culture.

Microbiological check

Checking the infectious markers of the donor

Viral genomic diagnosis is carried out in conjunction with the donor for HIV, and HBV and HCV. Compliant infectious markers are: HIV: Combined Ag P24 + Ac anti-HIV 1+2 test: Negative, PCR HIV: Negative, HBV: negative Ag HBs, negative Ac anti-HBc, PCR HBV: Negative, HCV: Ac

anti-HCV: Negative, PCR HCV: Negative, HTLV: Ac anti HTLV I+II: Negative, Syphilis: Ac anti-TP: Negative, CMV, EBV and Toxoplasmosis: negative IgM, negative or positive IgG.

Bacteriology

The microbiological check is carried out according to the recommendations of the National Drug Safety and Health Products Agency (ANSM) for Cell Therapy Products (CTP) from aerobic and anaerobic hemocultures produced using the Bactec technique. This check is carried out during each culturing step. On the thawed MSCs, the results of the tests obtained *a posteriori* from the injection of the MSCs into the patient must be negative. In the event of a positive microbiological check after reinjection, clinician information will be produced immediately, the identification of the seed and the results of the antibiogram will be sent to the MTI-PP department in accordance with the internal procedure.

Other checks

The absence of hTERT transcript

The absence of telomerase activity is sought by qRT-PCR in the MSCs after culturing (end of P1 and P3). This check will be carried out on a cryopreserved aliquot of 10^6 MSCs.

Karyotype

It is carried out on a fresh cell sample of MSC obtained at the end of culturing (end of P1 and P3), before cryopreservation.

Dosage of endotoxins and mycoplasmas

This is carried out on a fresh or frozen cell sample of MSC obtained at the end of culturing (end of P1 and P3), before cryopreservation.

Administration of the MSCs

The cells thus prepared are administered to hospital patients in resuscitation units exhibiting septic shock or sepsis at a dose of at least 1×10^6/kg heterologous MSCs in 75 mL of 4 % albumin, NaCl, ACD, infused for 30 minutes on the central venous line. The treatment is received and administered on the central venous line preferably within 10 hours following the step of thawing and washing the MSCs of the WCB, and at most up to 24 hours following the step of thawing and washing the MSCs of the WCB.

Murine model of septic shock

Septic shock was induced in immunocompetent C57BL/6 mice by ligation and perforation of the *coecum* (CLP). This model, which is considered to be the best standard for murine septic shock, allows human peritonitis to be imitated.

After surgery, the mice were randomized into 3 groups: a group receiving 0.25×10^6 MSCs of human WJ, a group receiving 0.25×10^6 MSCs of BM obtained by culturing from a bone marrow sample of a healthy donor having given their consent and a control group receiving PBS. The MSCs and the PBS were administered 24 hours after the onset of septic shock by the intravenous route on the retro-orbital sinus.

The WJ-MSCs and BM-MSCs are used immediately after thawing without prior re-culturing.

Example 2: Effect of WJ-MSCs on septic shock in mice

Impact of WJ-MSCs on bacteremia

Protocol

The thawed human WJ-MSCSs are used.

Forty-eight hours after the onset of septic shock, that is 24 hours after the administration of MSCs, the mice were killed by a lethal injection of anaesthetic. The spleen and blood of the mice were removed and then seeded in order to count the number of CFUs (Colony Forming Unit).

The various groups were compared using a Kruskal-Wallis test.

Results

Two days after septic shock, only the mice treated with WJ-MSCs showed a significant reduction in bacteremia and the number of splenic CFUs. The average number of CFUs per milligram of spleen or blood was counted. Forty-eight hours after the CLP procedure, the group treated with the WJ-MSCs exhibited an average of 2.3×10^3 aerobic CFU per mg of spleen and of 5.8×10^3 anaerobic CFU per mg of spleen, whereas the control group and the group treated with BM-MSC exhibited an average count of aerobic CFU per mg of spleen of 9.5×10^4 and of 5.5×10^3, respectively, and an average count of anaerobic CFU per mg of spleen of 1.3×10^5 and 1.1×10^4, respectively (**Figures 1A and 1B**).

The results obtained in the blood are similar (**Figure 2A and 2B**). The group treated with the MSCs derived from Wharton's jelly exhibited an average of 6×10^3 aerobic CFU per mL of blood

and of 2.1×10^4 anaerobic CFU per mL of blood, while the control group and the group treated by BM-MSC exhibited an average count of aerobic CFU per mL of blood of 1.3×10^7 and 1.1×10^5, respectively, and an average count of anaerobic CFU per mL of blood of 7.7×10^6 and 3.2×10^5, respectively.

Conclusions

These results show, for the first time, an antibacterial action of MSCs derived from Wharton's jelly in septic shock when they are used immediately after thawing.

Impact of WJ-MSCs on cell influx within organs

Protocol

Forty-eight hours or 7 days after the induction of septic shock, the mice were killed by an intra-peritoneal injection of pentobarbital and the organs were removed. The spleen and the liver were ground and then filtered. The bone marrow was extracted from the femur by rapidly injecting 1 ml of PBS into the medullary cavity. The lungs were cut into thin pieces and then placed in 2 ml of collagenase for 45 minutes before being ground and filtered. The cells extracted from the various organs were washed by centrifugation. A cell count was carried out.

The cells were marked with 5 µL of anti-CD45, CD11b, Ly6C, Ly6G antibodies in order to identify and quantify the total monocytes, the inflammatory and anti-inflammatory monocytes, as well as the neutrophils, within the various organs.

The same protocol was implemented on healthy mice before the induction of septic shock.

The various groups were compared using a 2-way ANOVA test, followed by a Tukey test. A statistical significance was accepted for $p < 0.05$.

Results

Two days after the induction of septic shock, the control mice that intravenously received a saline solution exhibited a significantly greater number of lung neutrophils than the mice treated by injecting mesenchymal stem cells (**Figure 3A**). On D7 a significantly lower accumulation of neutrophils was noted on the spleens and livers of the mice treated with BM-MSC and WJ ($p < 0.001$) (**Figures 3B, 3C**). Seven days after CLP the number of neutrophils contained in the femurs of the treated animals was significantly lower compared to the control group (PBS vs BM $p < 0.05$; PBS vs WJ $p < 0.01$) (**Figure 3D**).

Two days after the induction of septic shock and 24 hours after injecting the MSCs, the lungs of the mice that received BM- or WJ-MSC contained a significantly lower number of ly6C^{high} inflammatory monocytes than in the control mice ($p < 0.01$) (**Figure 4A**). A significant reduction in the number of total monocytes (**Figure 4B**) and the number of ly6C^{high} inflammatory monocytes (**Figure 4C**) on D2 was also found on the femurs of mice treated with WJ-MSCs ($p < 0.05$) compared to the control mice, whereas no significant difference was noted between the control group and the BM-MSC group.

Seven days after the induction of septic shock a significantly lower number of ly6C^{high} inflammatory monocytes was observed in the spleens of the mice treated with WJ-MSCs compared to the mice treated with BM-MSC ($p < 0.01$) and with PBS (< 0.05) (**Figure 4D**).

This study demonstrates that the MSCs are capable of modulating leukocyte infiltrate in a polymicrobial sepsis model. It can be seen that the action of the thawed WJ-MSCs is not limited to the first 48 hours following their injection, but that they are able to have much more delayed effects on cell recruitment during septic shock.

In addition to being effective at a late stage, the MSCs also exhibit an early action on leukocyte traffic. The results analysed above show that MSCs decrease the influx of neutrophils into the lungs 48 hours after the induction of septic shock.

These results demonstrate that WJ-MSCs, like BM-MSCs, are capable of reducing accumulation within neutrophil organs, involved in the development of organ failures associated with septic shock. Indeed, it is known that the abnormal accumulation of neutrophils can induce, on the one hand, a vascular occlusion leading to hypoxemia and tissue hypoperfusion and, on the other hand, can generate microcirculation dysfunctions by a massive release of reactive oxygen species.

Furthermore, unlike the BM-MSCs, the WJ-MSCs are capable of reducing, on the one hand, the femoral production of total monocytes two days after septic shock and, on the other hand, the production of pro-inflammatory monocytes leading to less accumulation of this monocyte sub-population in the spleen of mice. Since the septic shock changes the properties of monocytes by particularly inducing an increase in their production of reactive oxygen species resulting in an increase in the SOFA score (Sepsis-related Organ Failure Assessment) (Martins *et al.*, 2008), the

reduction in the accumulation and production of the total and pro-inflammatory monocytes induced by injecting WJ-MSCs can provide a real benefit in the treatment of septic shock.

Impact of WJ-MSCs on survival

Protocol

After the induction of septic shock in C57B1/6 mice using the CLP technique, 150 µl of NaCl was subcutaneously administered in order to allow vascular filling after surgery. For the purpose of more closely representing clinical conditions, all the mice received a dose of 50 µg/g of body weight of imipenem every 12 hours by the intraperitoneal route (Alcayaga-Miranda *et al.*, 2015).

A Wilcoxon test was carried out. A significant difference has been acknowledged for $p < 0.05$.

Results

The survival rate has increased in MSC-treated mice compared to the control mice. A 64 % survival rate is found in the control animals, whereas 83 % of the animals treated with BM-MSCs and 87 % of the animals treated with WJ-MSCs survived (**Figure 5**).

The WJ-MSCs exhibit better results in terms of survival in septic shock compared to the BM-MSCs.

Example 3: Study of the impact of WJ-MSCs on sepsis

The efficacy of the clinical grade thawed WJ-MSCs produced according to the method described in section 1.1-1.6 above is analysed in pigs, which is the animal closest to a human from a cardiovascular perspective.

Four hours after the induction of peritonitis in pigs, a 1×10^6/kg dose of WJ-MSCs was injected intravenously. The WJ-MSCs were produced as clinical grade and were used immediately after thawing. The study, carried out over 24 hours following the induction of peritonitis, was carried out double blind and in the presence of an experienced resuscitation doctor. Accordingly, the support was identical to that of a patient with maintenance, on the one hand, of the volemia and the mean arterial pressure (> 85 mmHg) by vascular filling and noradrenaline (maximum 10 µg/kg/min) and, on the other hand, by an adequate cardiac flow (> 21/min/m^2) by dobutamine (maximum 20 µg/kg/min).

This study demonstrates that administering WJ-MSCs significantly improves the venous oxygen

saturation exhibiting better suitability between the O_2 contributions and the O_2 consumption in the treated animals (**Figure 6A**).

Administering WJ-MSCs improves the cardiovascular functions as demonstrated by the improvement of the mean arterial pressure (MAP) in the treated animals and the later administration of noradrenaline (**Figures 6B, 6C**). Administering WJ-MSCs improves the renal function: the treated animals exhibited a lower increase in creatinine and greater diuresis (**Figures 6D, 6E**).

Furthermore, the intravenous injection of WJ-MSCs significantly reduces lactatemia, which when more than 2 mmol/l is produced indicates tissue hypoxia (**Figure 6F**).

Intravenous injection of WJ-MSCs also increases the ratio PaO_2:FiO_2, which reflects the intensity of acute respiratory distress syndrome (**Figure 6G**). Its increase in treated animals demonstrates less pulmonary failure compared to untreated animals.

This experiment has shown that the increase in the survival of treated animals compared to untreated animals is approximately 60 % (**Figure 6H**).

Example 4: Analysis of obstetric factors

50 umbilical cords were analysed by combining various obstetric parameters. The WJ-MSCs are isolated from these tissues in accordance with the method described in section 1.1-6 above.

After the data are extracted, the 27 obstetric factors (14 relating to the mother, 6 to the new-born and 7 to the childbirth) are analysed for any correlations between these obstetric parameters and 8 biological indicators of cell proliferation (doubling of population). Each variable has undegrone a bivariate linear regression analysis (BLRA). Only the factors exhibiting a significant association with the threshold of 0.15 in the BLRA were candidates for a multivariate linear regression analysis (MLRA). The method for selecting Stepwise variables was used with a 0.1 input threshold into the model and a 0.05 output threshold from the model.

Following a multivariate linear regression analysis, the administration of oxytocin at the time of childbirth demonstrated a positive impact on the proliferation of WJ-MSCs by decreasing the doubling time (61.6 ± 5.2 hours vs 112.0 ± 19.5 hours, $p = 0.0159$) during the first passage P1 (**Figure 7A**). This administration of oxytocin also allowed a greater number of cells to be obtained

with a short doubling time (< 100 hours) (57.9 % vs. 25 %, p = 0.0469). By only considering the samples with a doubling time during P1 of less than 100 hours, several factors exhibited a positive impact on the doubling time: directed labour (34.2 % vs 0 %, p = 0.0185), the number of weeks of amenorrhea at childbirth (39.85 vs 37.92, p = 0.0212), maternal smoking (42.1 % vs 8.3 %, p = 0.0313) and the weight of the placenta (552.24 vs 481.92, p = 0.0446). For the second passage (P2), the weight at birth, the number of weeks of amenorrhea, the weight of the placenta, a normal pregnancy and an absence of preeclampsia demonstrated a positive impact on cell proliferation (**Figure 7B**). All these factors are related to the notion of birth at full term and demonstrate that WJ-MSCs, originating from new-borns in good health and born at full term, will exhibit superior proliferative capacities. It also has been noted that WJ-MSCs derived from umbilical cords of smoking mothers exhibit increased proliferative capacities.

The identification of these factors promoting the proliferation of WJ-MSCs assists the selection of umbilical cords that contain WJ-MSCs with better proliferation properties.

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Patentkrav

1. Tinte humane mesenkymale stamceller (MSC-er) fra Whartons gelé for anvendelse i behandling av sepsis, særlig av septisk sjokk; de tinte humane MSC-ene fra Whartons gelé er **karakterisert ved at**:

deres ekspresjonsnivå av CD44 er minst 20 % lavere enn ekspresjonsnivået av CD44 i ferske humane MSC-er fra Whartons gelé; eller

deres nivå av CD90, CD73, CD105, CD146 eller CD166 er minst 10 % lavere enn nivået av henholdsvis CD90, CD73, CD105, CD146, eller CD166, i ferske humane MSC-er fra Whartons gelé.

2. De tinte humane MSC-ene fra Whartons gelé for anvendelse ifølge krav 1, **karakterisert ved at** ekspresjonsnivået til minst én markør valgt fra CD90, CD73, CD105, CD29, CD146, CD166, HLA-ABC er minst 10 % lavere enn ekspresjonsnivået til den samme markøren i ferske humane MSC-er fra Whartons gelé.

3. De tinte humane MSC-ene fra Whartons gelé for anvendelse ifølge krav 1 eller 2, **karakterisert ved at** MSC-ene uttrykker minst ett protein valgt fra gruppen omfattende ACTB, ANXA1, CAPZB, LASP1, PRDX2, PRDX3, PSA3, RS12 og SYWC.

4. De tinte humane MSC-ene fra Whartons gelé for anvendelse derav ifølge et hvilket som helst av kravene 1 til 3, **karakterisert ved at** MSC-ene i det vesentlige ikke uttrykker minst ett protein valgt fra gruppen omfattende ACTS, AL1B1, ANX10, GBB1, GBB2, GPRIN1, DTNA, MIPO1, PSB3 og PSDE.

5. De tinte humane MSC-ene fra Whartons gelé for anvendelse derav ifølge et hvilket som helst av kravene 1 til 4, **karakterisert ved at** MSC-ene, under *in vitro*-tilstand og/eller under ikke-inflammatorisk tilstand, utskiller minst én vekstfaktor valgt fra BMP-7, IGFBP-1, insulin, FGF-7, NT-4 og VEGF-D.

6. De tinte humane MSC-ene fra Whartons gelé for anvendelse derav ifølge et hvilket som helst av kravene 1 til 5, **karakterisert ved at** MSC-ene, under *in vitro*-tilstand og/eller under ikke-inflammatorisk tilstand, utskiller minst én vekstfaktor valgt fra BMP-7 og TGFβ3.

7. De tinte humane MSC-ene fra Whartons gelé for anvendelse derav ifølge et hvilket som helst av kravene 1 til 6, **karakterisert ved at** MSC-ene, under *in vitro*-tilstand og/eller under inflammatorisk tilstand, i det vesentlige ikke utskiller IGFBP-1.

8. Den tinte humane MSC-en fra Whartons gelé for anvendelse derav ifølge et hvilket som helst av kravene 1 til 7, **karakterisert ved at** cellene er avledet fra et humant navlestrengsvev fra en mor som oppfyller minst ett av de følgende kriteriene: ha mottatt en administrering av oksytocin under fødselen, ha født ved tilrettelagt fødsel, ha født til termin, ikke hatt svangerskapsforgiftning under graviditeten, hvis barn ikke har hatt neonatale lidelser og har vært utsatt for et inntak av tobakksrøyk under graviditeten.

9. De tinte humane MSC-ene fra Whartons gelé for anvendelse ifølge et hvilket som helst av kravene 1 til 8, **karakterisert ved at** MSC-ene er celler av klinisk kvalitet.

10. De tinte humane MSC-ene fra Whartons gelé for anvendelse ifølge et hvilket som helst av kravene 1 til 9, **karakterisert ved at** cellene er oppnådd direkte fra tining uten redyrking etter tining.

11. Farmasøytisk sammensetning omfattende tinte humane mesenkymale stamceller fra Whartons gelé ifølge et hvilket som helst av kravene 1 til 10 og et farmasøytisk akseptabelt hjelpestoff, for anvendelse i behandling av sepsis.

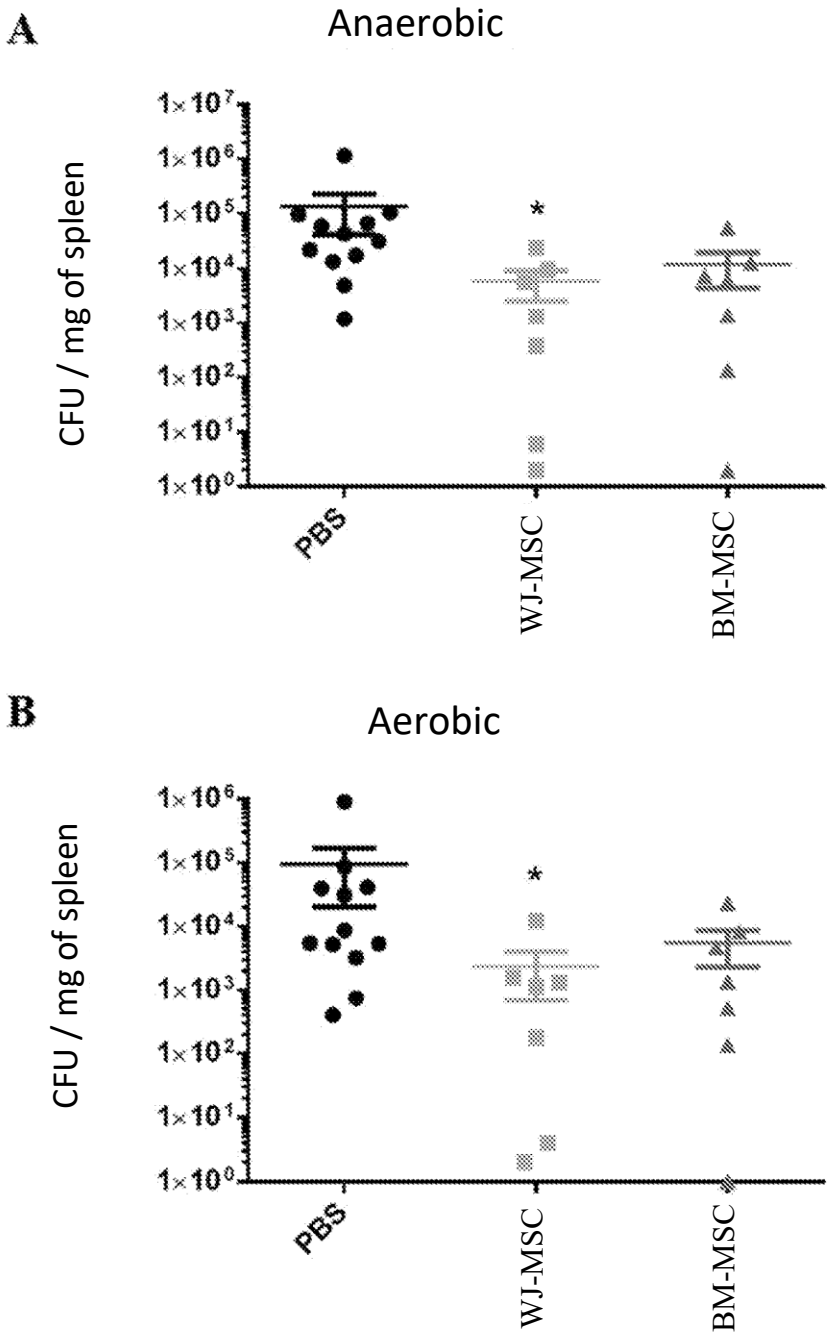

A
Anaerobic
CFU / mg of spleen
1×10^7
1×10^6
1×10^5
1×10^4
1×10^3
1×10^2
1×10^1
1×10^0
*
PBS
WJ-MSC
BM-MSC
B
Aerobic
CFU / mg of spleen
1×10^6
1×10^5
1×10^4
1×10^3
1×10^2
1×10^1
1×10^0
*
PBS
WJ-MSC
BM-MSC

FIG. 1

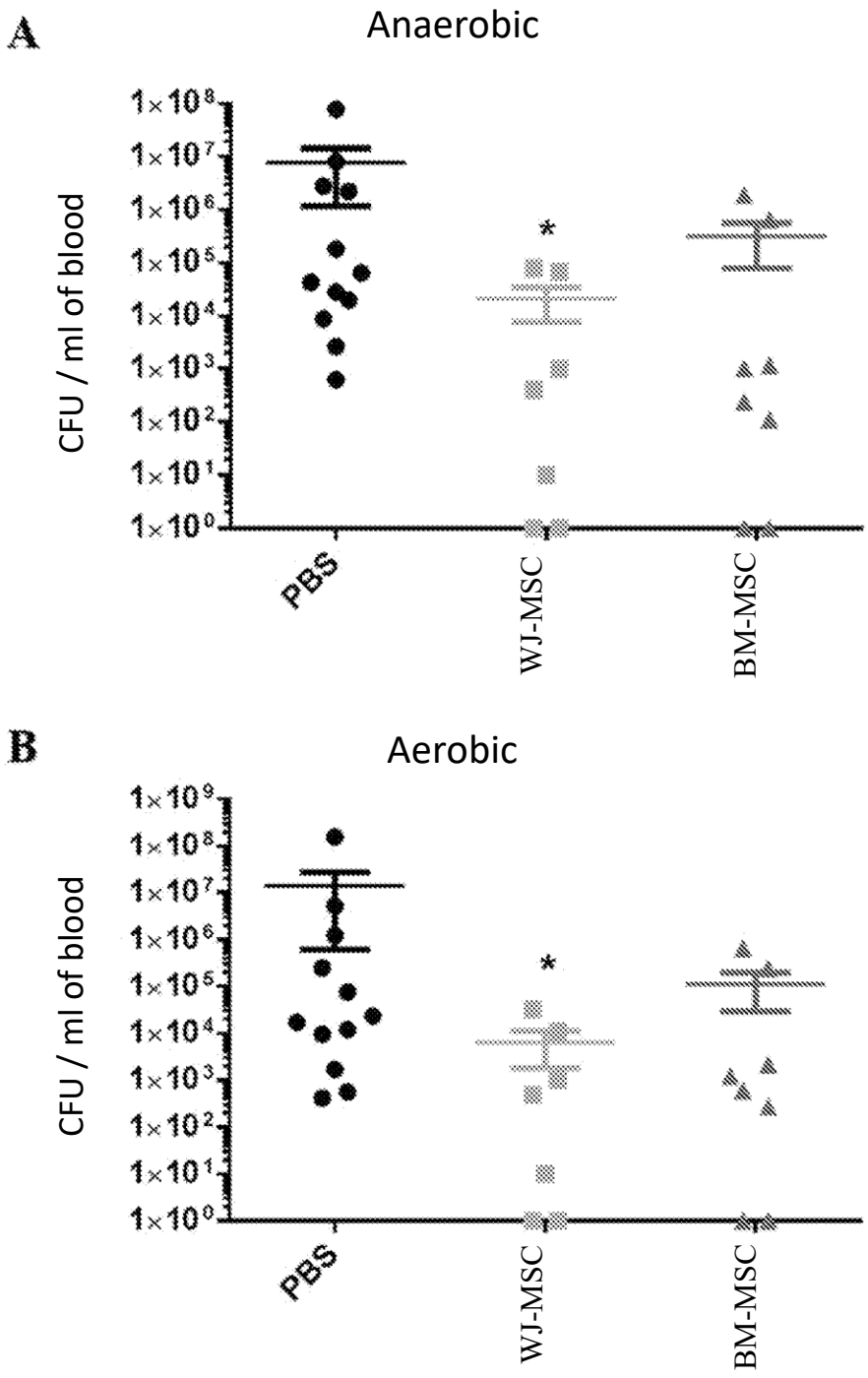
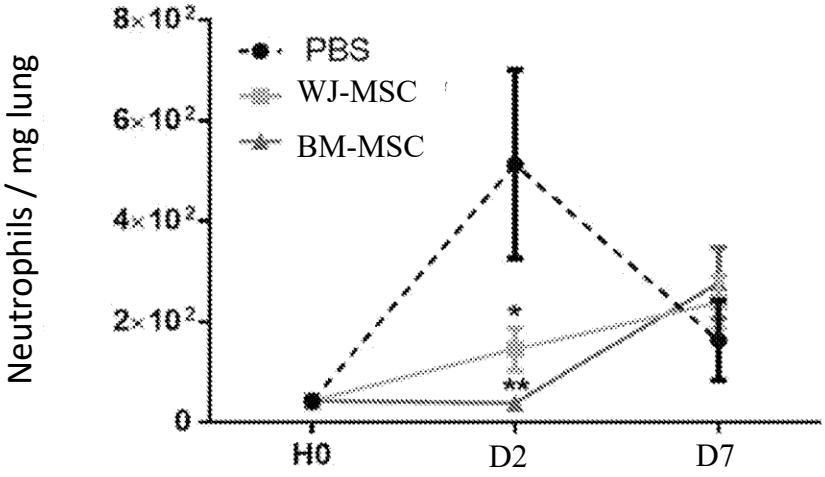

A
Anaerobic
CFU / ml of blood
1×10^8
1×10^7
1×10^6
1×10^5
1×10^4
1×10^3
1×10^2
1×10^1
1×10^0
*
PBS
WJ-MSC
BM-MSC
B
Aerobic
CFU / ml of blood
1×10^9
1×10^8
1×10^7
1×10^6
1×10^5
1×10^4
1×10^3
1×10^2
1×10^1
1×10^0
*
PBS
WJ-MSC
BM-MSC

FIG. 2

A


Neutrophils / mg lung
8×10²
6×10²
4×10²
2×10²
0
PBS
WJ-MSC
BM-MSC
*
**
H0
D2
D7

B

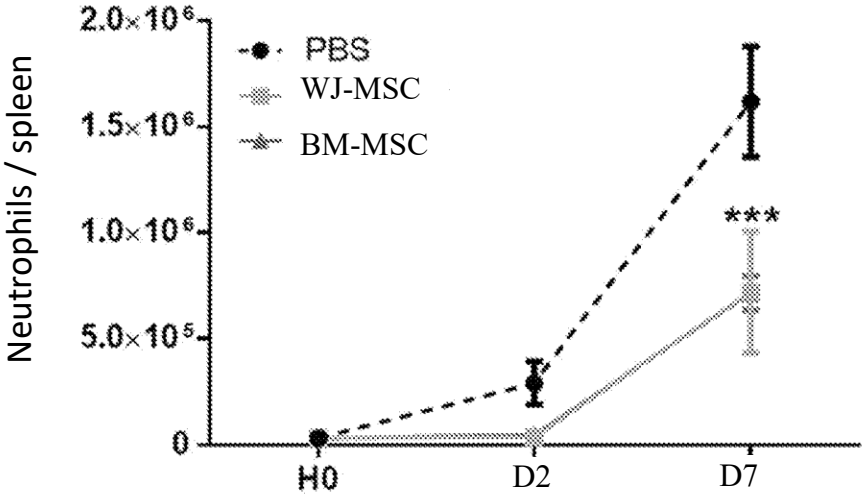

Neutrophils / spleen
2.0×10⁶
1.5×10⁶
1.0×10⁶
5.0×10⁵
0
PBS
WJ-MSC
BM-MSC
***
H0
D2
D7

FIG. 3 A-B

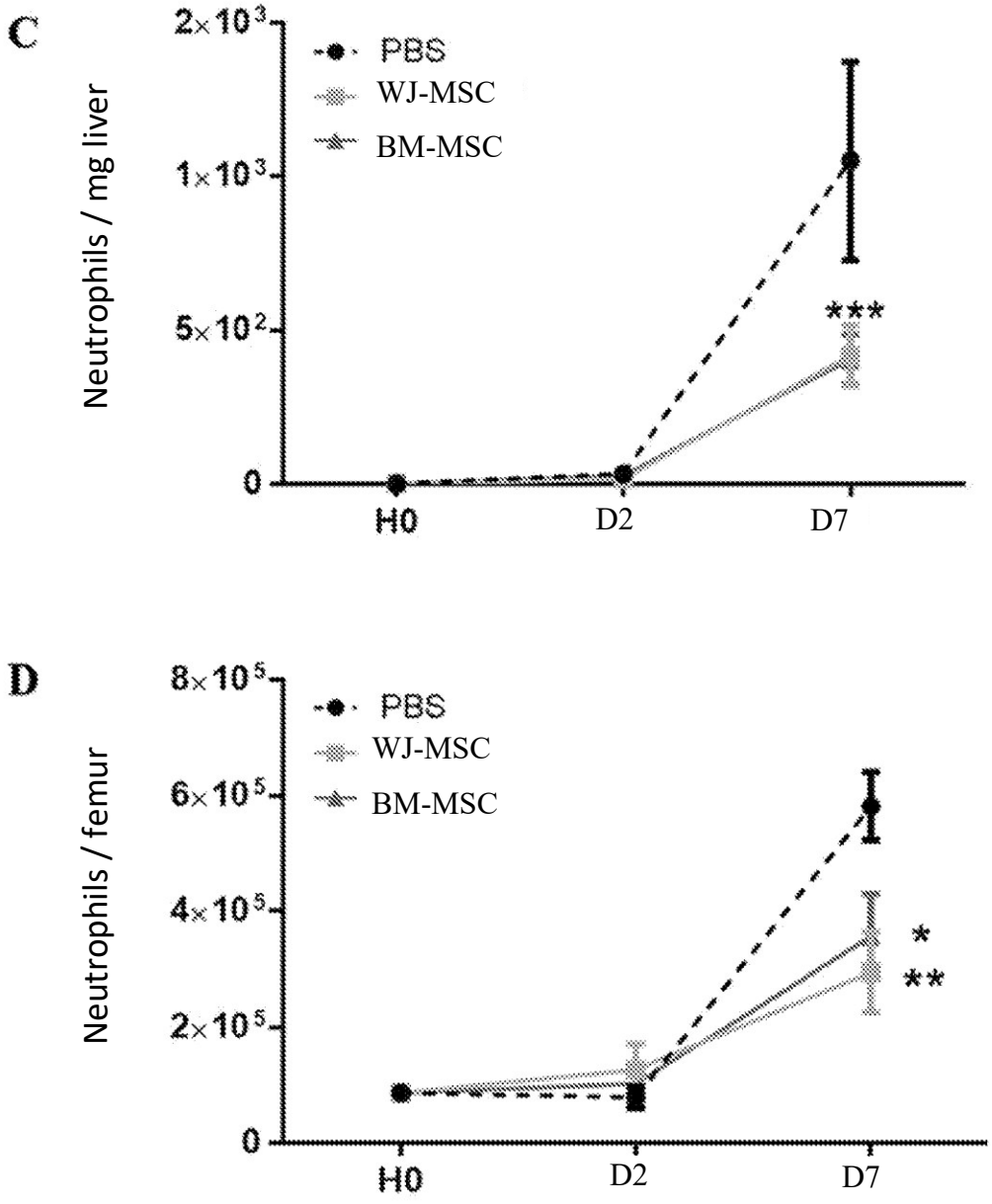

C
Neutrophils / mg liver
2×10³
1×10³
5×10²
0
PBS
WJ-MSC
BM-MSC
***
H0
D2
D7
D
Neutrophils / femur
8×10⁵
6×10⁵
4×10⁵
2×10⁵
0
PBS
WJ-MSC
BM-MSC
*
**
H0
D2
D7

FIG. 3 C-D

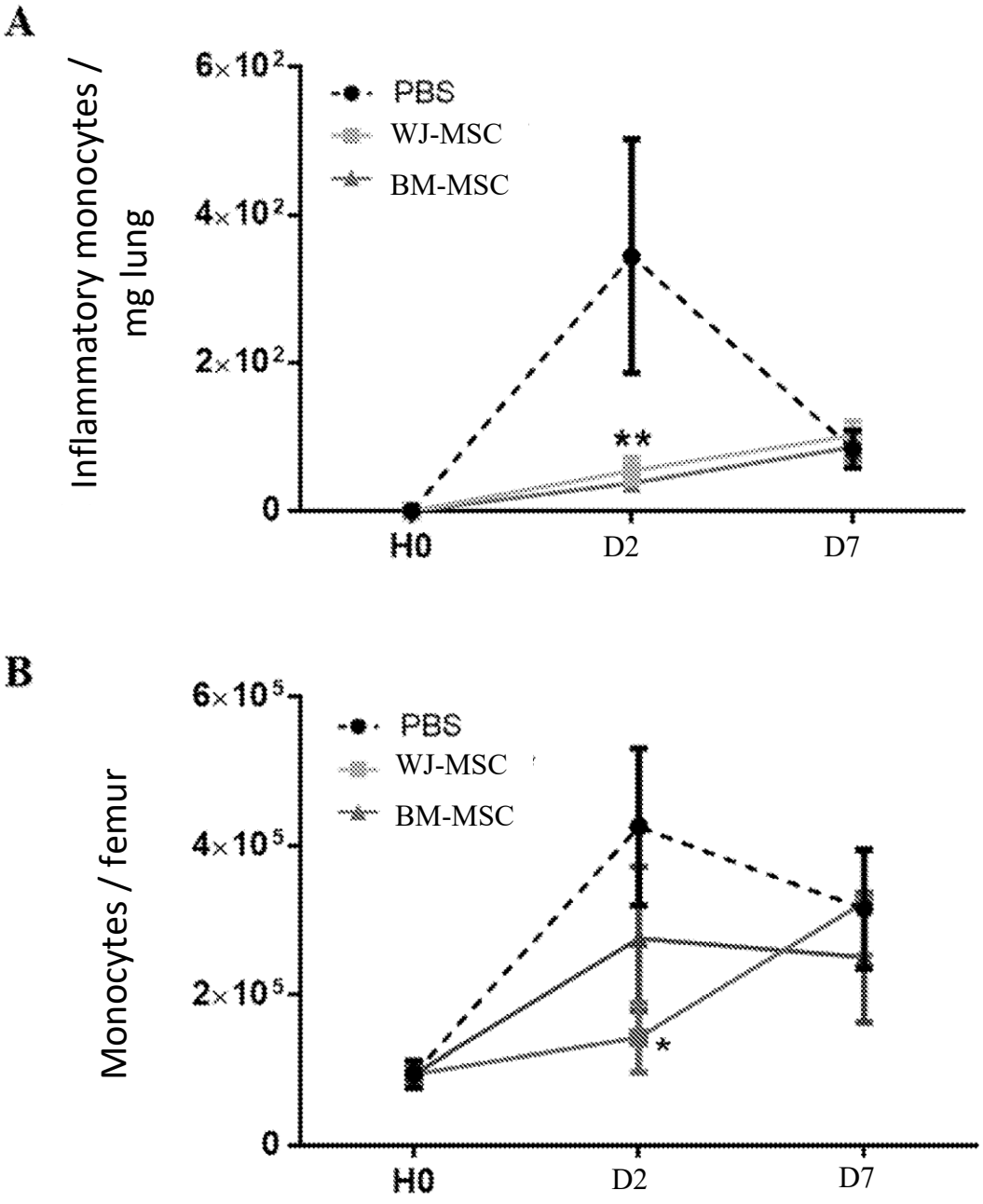

A
Inflammatory monocytes / mg lung
6×10²
4×10²
2×10²
0
PBS
WJ-MSC
BM-MSC
**
H0
D2
D7
B
Monocytes / femur
6×10⁵
4×10⁵
2×10⁵
0
PBS
WJ-MSC
BM-MSC
*
H0
D2
D7

FIG. 4 A-B

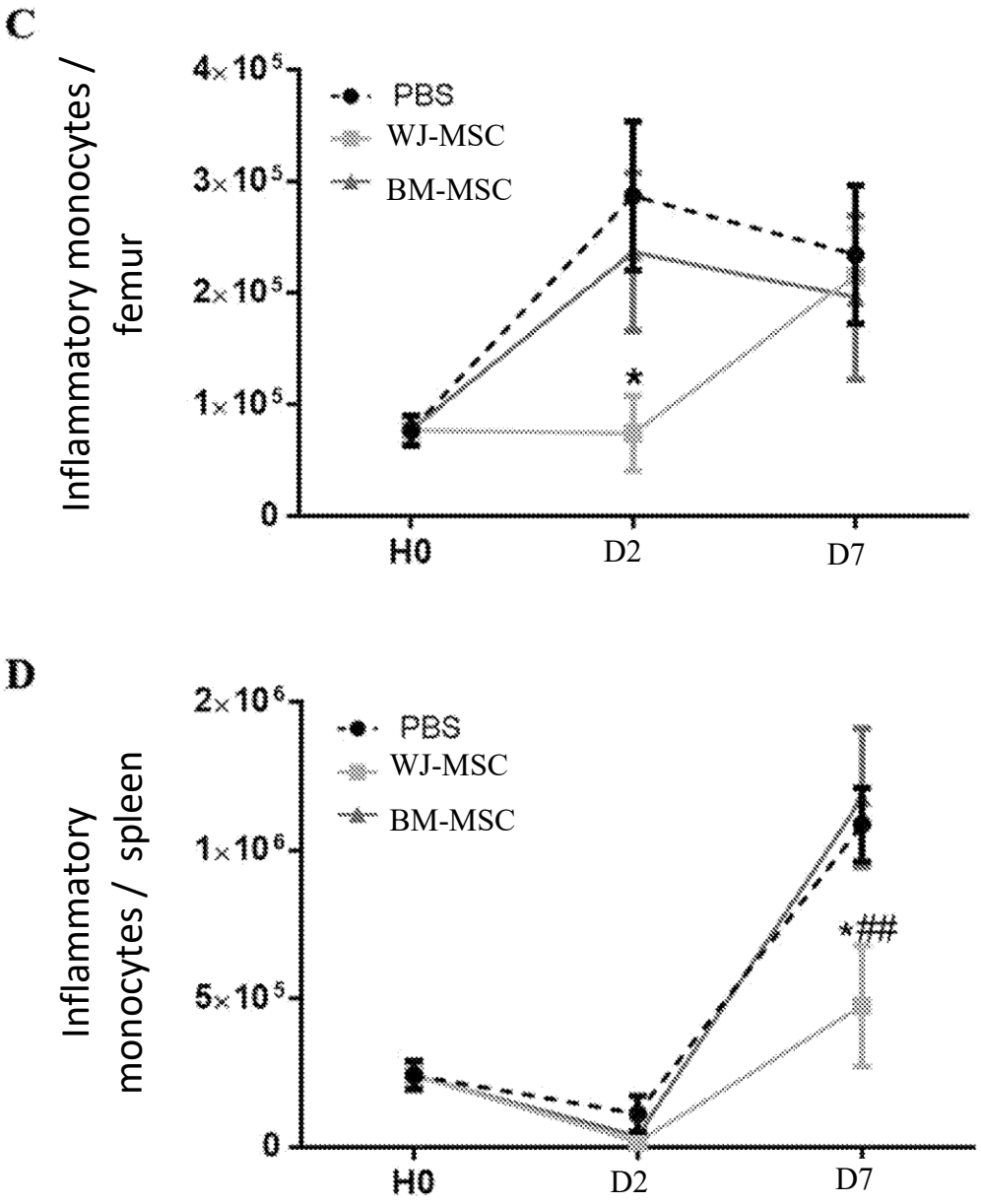

C
Inflammatory monocytes / femur
4×10^5
3×10^5
2×10^5
1×10^5
0
PBS
WJ-MSC
BM-MSC
*
H0
D2
D7
D
Inflammatory monocytes / spleen
2×10^6
1×10^6
5×10^5
0
PBS
WJ-MSC
BM-MSC
*##
H0
D2
D7

FIG. 4 C-D

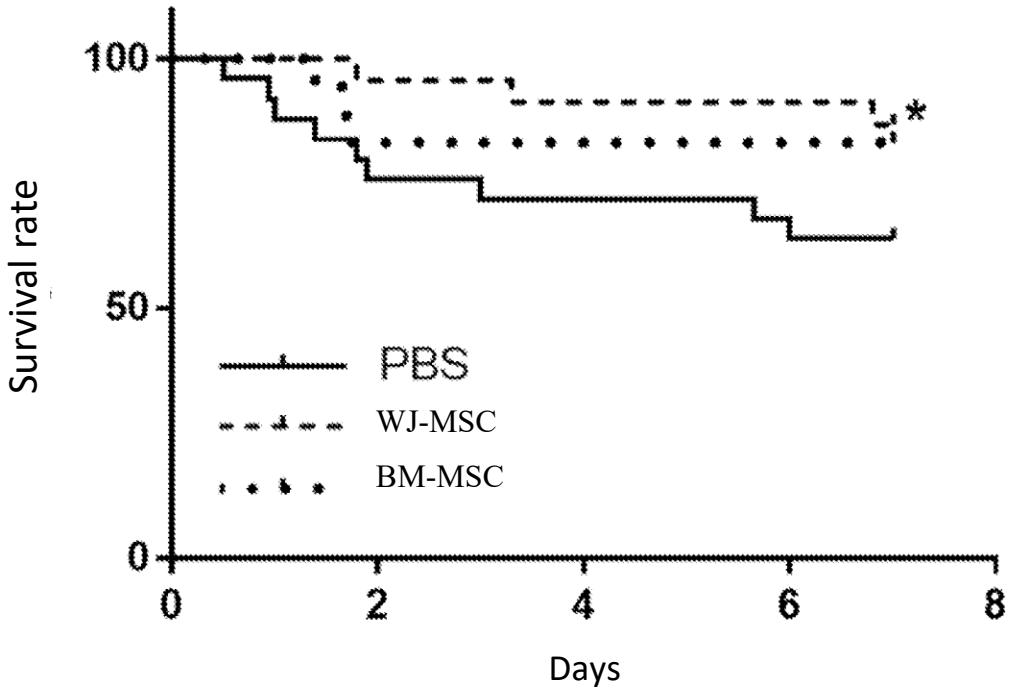


FIG. 5

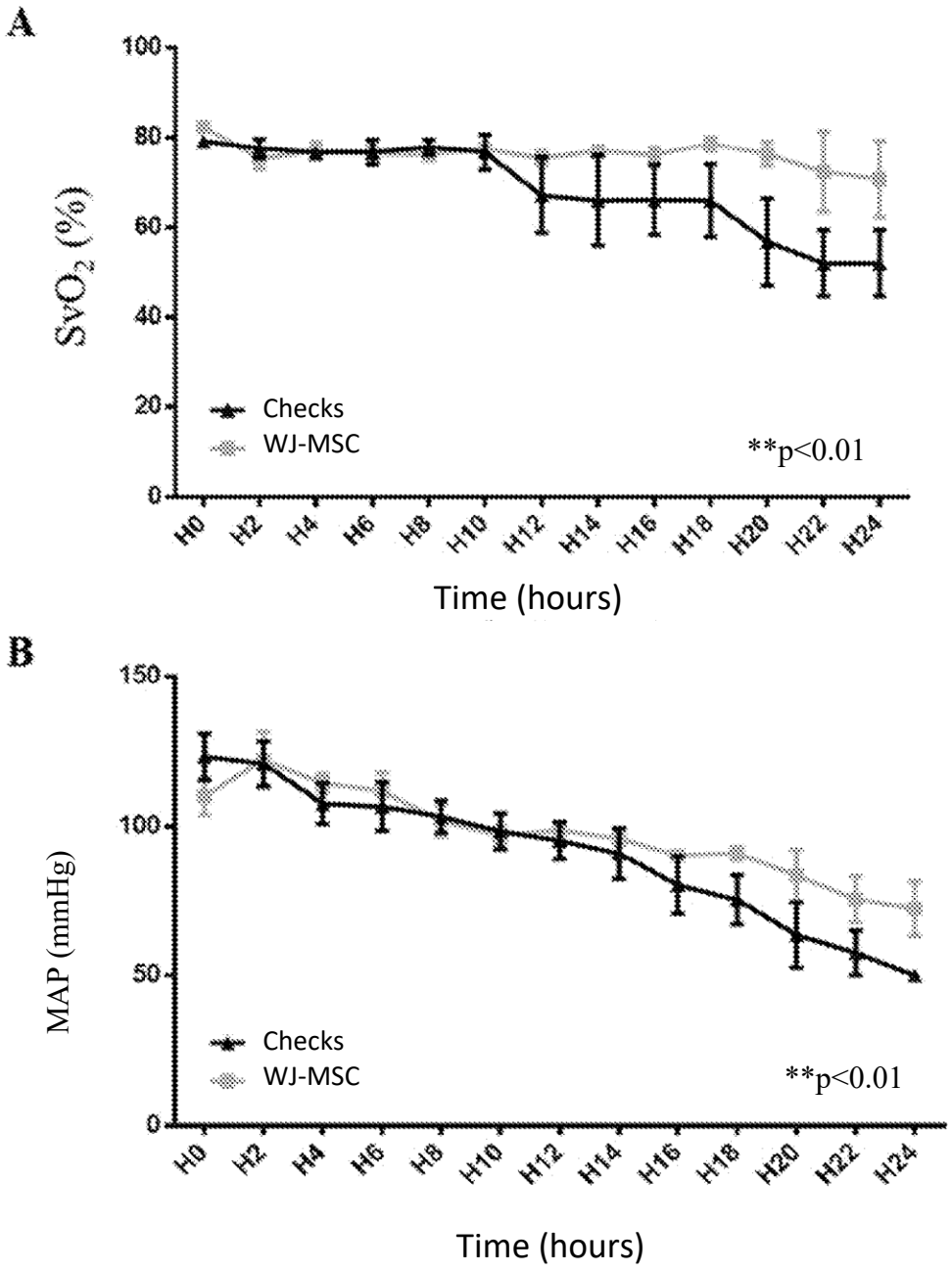

A
100
80
60
40
20
0
SvO2 (%)
Checks
WJ-MSC
**p<0.01
H0
H2
H4
H6
H8
H10
H12
H14
H16
H18
H20
H22
H24
Time (hours)
B
150
100
50
0
MAP (mmHg)
Checks
WJ-MSC
**p<0.01
H0
H2
H4
H6
H8
H10
H12
H14
H16
H18
H20
H22
H24
Time (hours)

FIG. 6 A-B

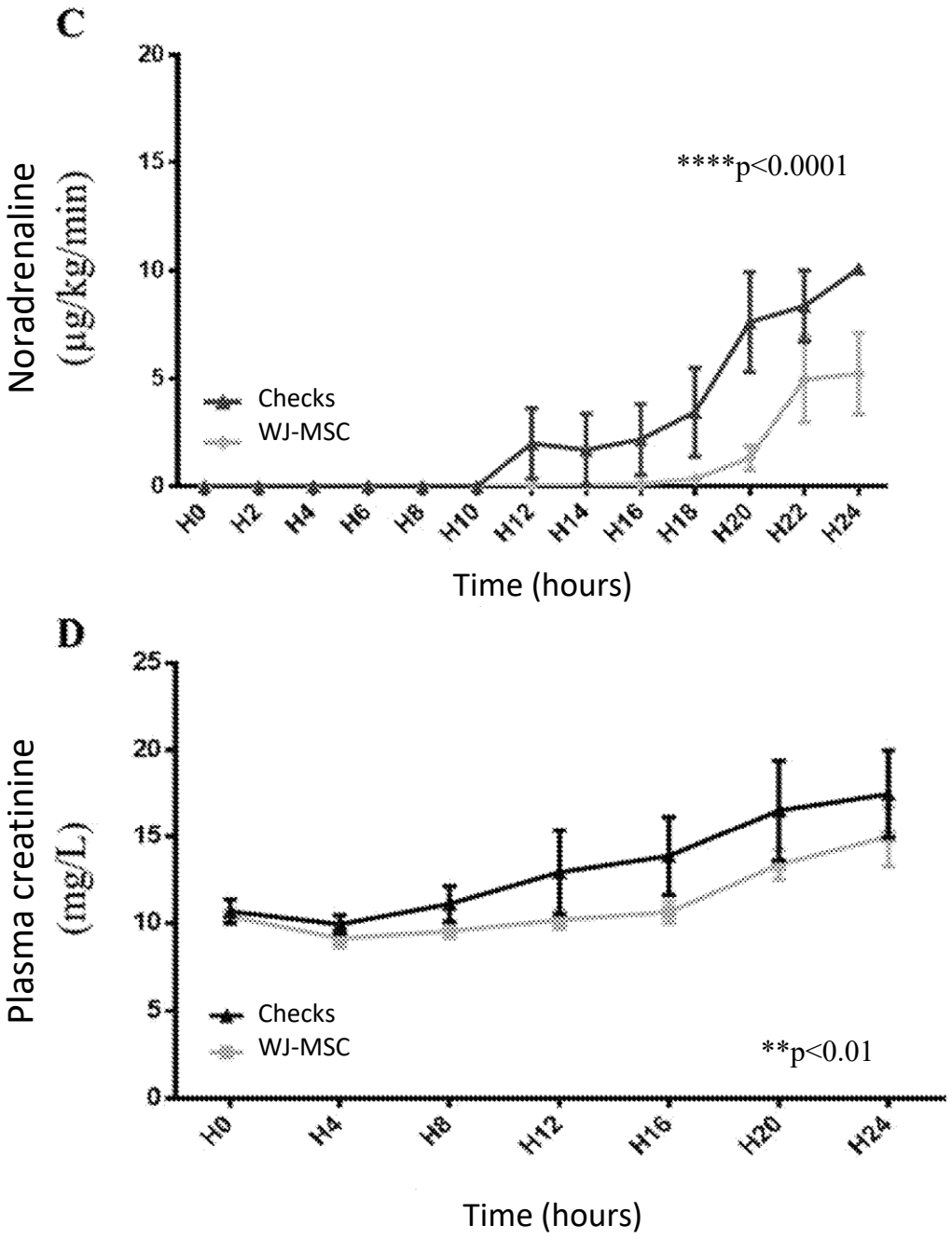

C
Noradrenaline (μg/kg/min)
20
15
10
5
0
****p<0.0001
Checks
WJ-MSC
H0
H2
H4
H6
H8
H10
H12
H14
H16
H18
H20
H22
H24
Time (hours)
D
Plasma creatinine (mg/L)
25
20
15
10
5
0
Checks
WJ-MSC
**p<0.01
H0
H4
H8
H12
H16
H20
H24
Time (hours)

FIG. 6 C-D

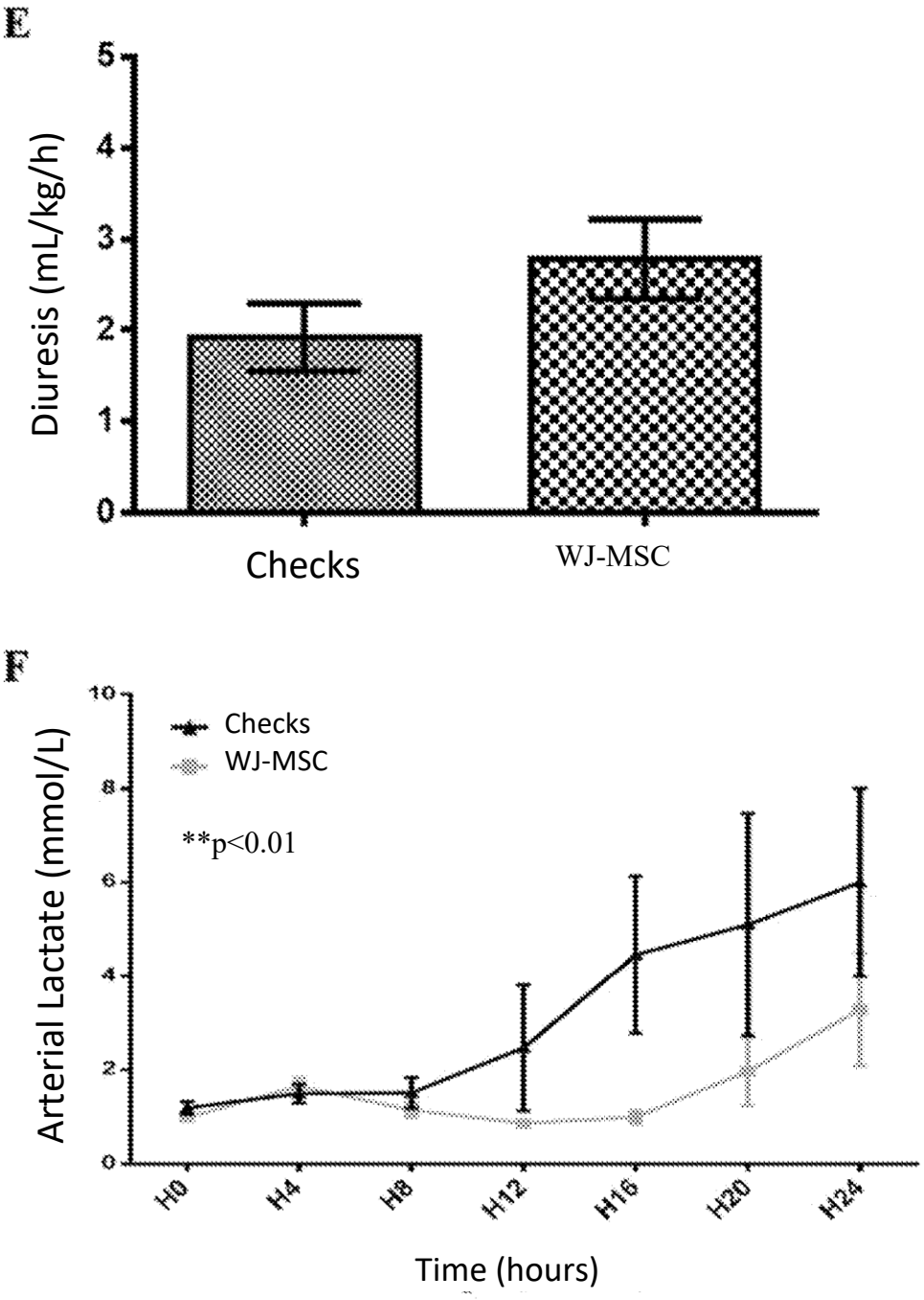

E
Diuresis (mL/kg/h)
0
1
2
3
4
5
Checks
WJ-MSC
F
Arterial Lactate (mmol/L)
Checks
WJ-MSC
**p<0.01
0
2
4
6
8
10
H0
H4
H8
H12
H16
H20
H24
Time (hours)

FIG. 6 E-F

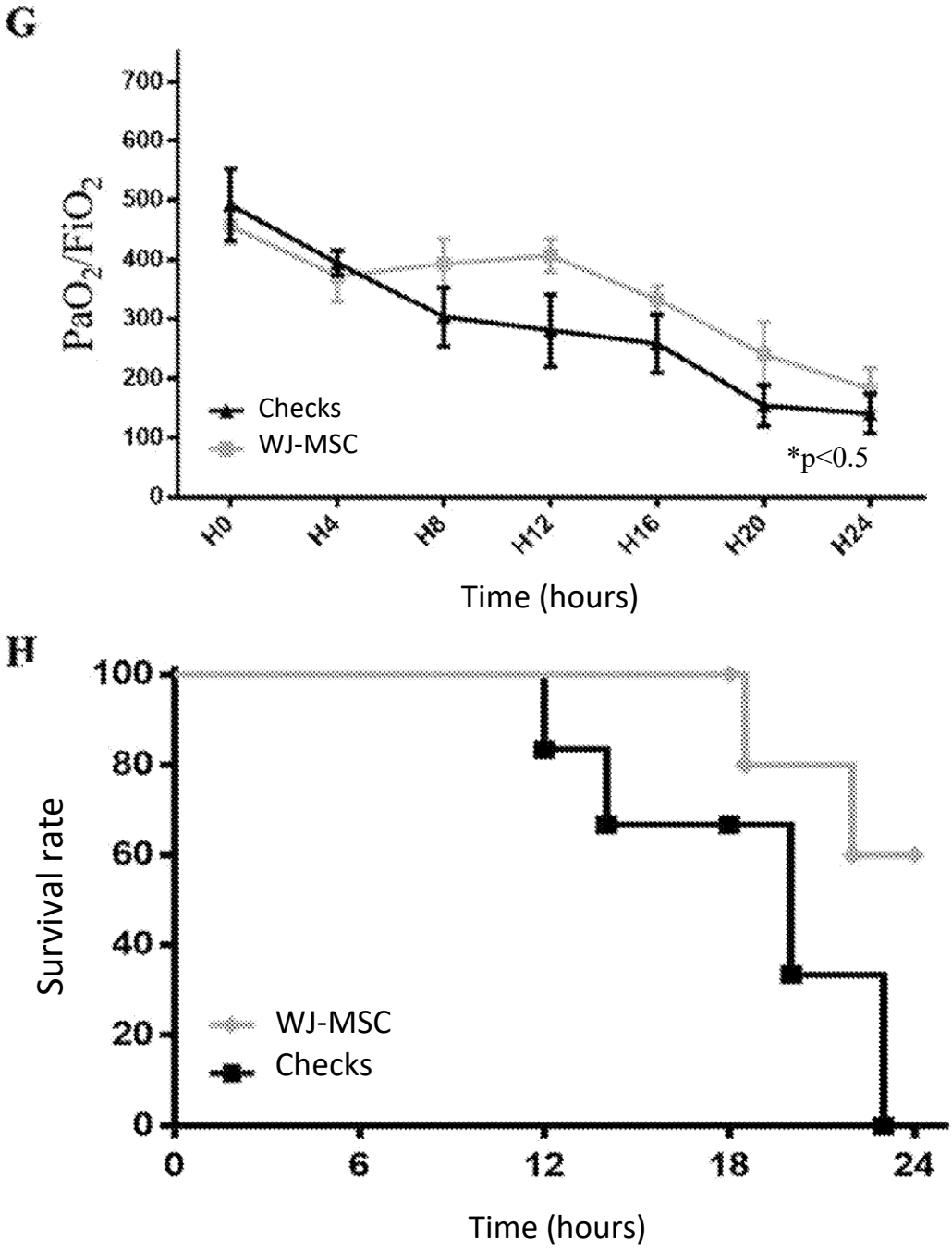

G
700
600
500
400
300
200
100
0
PaO2/FiO2
Checks
WJ-MSC
*p<0.5
H0
H4
H8
H12
H16
H20
H24
Time (hours)
H
100
80
60
40
20
0
Survival rate
WJ-MSC
Checks
0
6
12
18
24
Time (hours)

FIG. 6 G-H

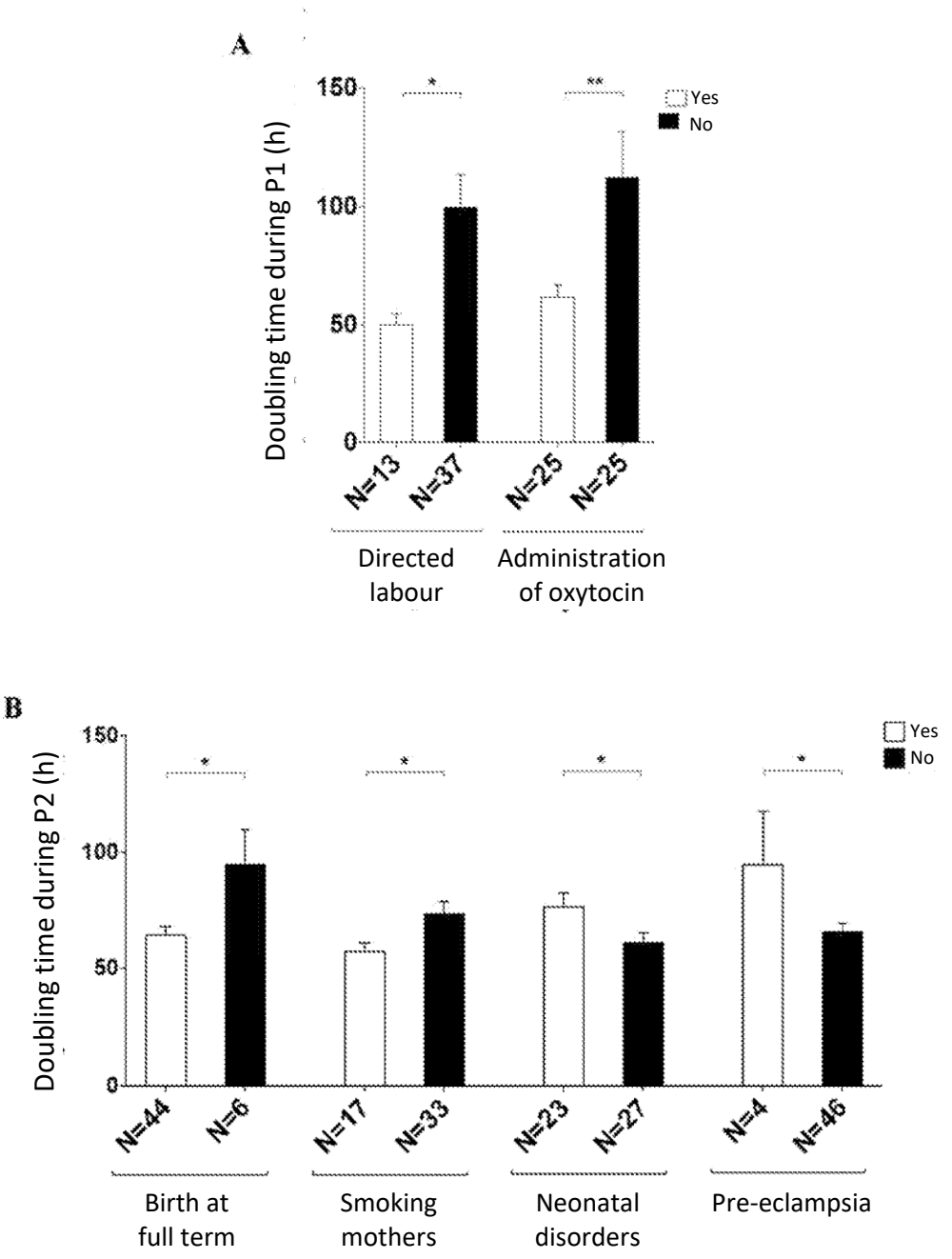

A
Doubling time during P1 (h)
150
100
50
0
*
**
Yes
No
N=13
N=37
N=25
N=25
Directed labour
Administration of oxytocin
B
Doubling time during P2 (h)
150
100
50
0
*
*
*
*
Yes
No
N=44
N=6
N=17
N=33
N=23
N=27
N=4
N=46
Birth at full term
Smoking mothers
Neonatal disorders
Pre-eclampsia

FIG. 7