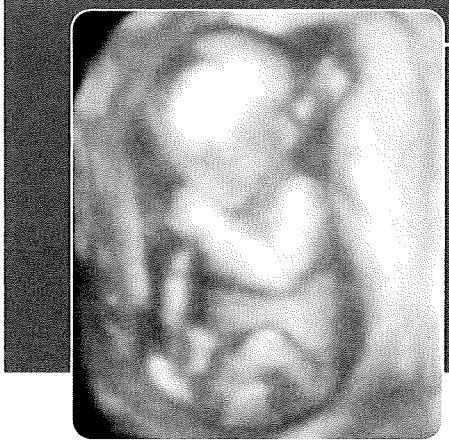

光環境が早産児・新生児の脳に与える影響

—新しい光受容体「メラノプシン」のもつ意味—

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はじめに

赤ちゃんの視覚に対する理解は、メラノプシン(melanopsin)とよばれる新しい光受容体の発見によって大きく変わろうとしている。光受容体とは、光(光子)を捕まえるタンパク質のことで、おもに目の網膜に存在する。メラノプシンは、最近の「生物時計」の研究を通して発見され、メラノプシンを含む節細胞は、桿体・錐体細胞に次ぐ第3の光センサーであることが明らかになった。

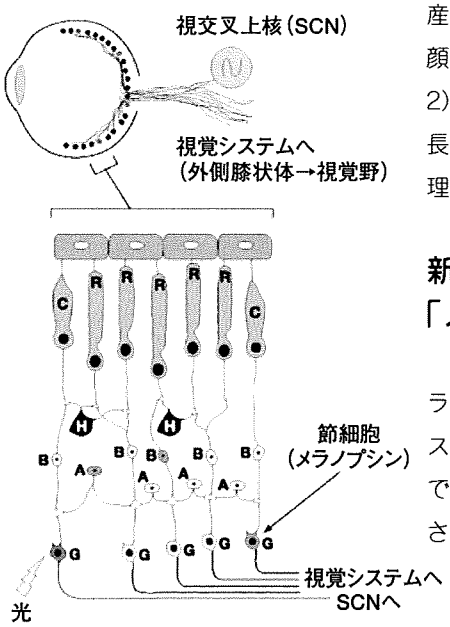

図1　網膜の視覚回路

メラノプシンを含む節細胞は、桿体・錐体細胞を経由せず、光に直接反応し視神経を通して光情報を生物時計に伝える。節細胞は桿体・錐体細胞からの入力も受けている。R(rod cell):桿体細胞、C(cone cell):錐体細胞、H (horizontal cell):水平細胞、B(bipolar cell):双極細胞、A(amacrine cell):アマクリン細胞、G(ganglion cell):節細胞。文献2より引用改変。

メラノプシンの最大の特長は、明暗情報の処理(明るい暗いの知覚)を行うことである。これは、以前から知られていたロドプシン(桿体細胞)・コーンオプシン(錐体細胞)といった光受容体が映像情報の処理(形・色の知覚)を行うのと対照的である。メラノプシンのもう1つの特長は、ロドプシン・コーンオプシンより早い発達段階で働き始めることである。この点は赤ちゃんの生後発達と視覚環境を考えるうえでとくに重要な意味をもっている。

メラノプシンの発見により、1)早産児と満期出産児の間でお母さんの顔を見分ける能力に差があること、2)光環境が早産児・新生児の体の成長に影響する可能性、をより明確に理解できるようになった。

新しい光センサー「メラノプシン」の発見

現在の視覚システムの理解は、メラノプシンの発見[1]によって新しいステージに入った。つい10年前まで、脊椎動物の視覚機能はほぼ解明されたと考えられていた。網膜外側に位置する桿体・錐体細胞がまず光を検出し、網膜内側の節細胞から視神経を通して外側膝状体を経由、最終的に脳(大脳皮質視覚野)に伝達、映像が認識されるという流れである(図1)。さらに、分子レベルでは、桿体細胞にはロドプシン、錐体細胞にはコーンオプシンという光受容体が存在し、光を11-シス型レチナール(11-cis-retinaldehyde)という分子で捕まえ、光を、生化学信号に変換、最終的には電気信号に変えて視神経へ伝達する。また、桿体細胞は弱い光にも反応し白黒の区別をするものの、詳細な映像を脳に伝達することはできない。錐体細胞は弱い光には反応しないが、青・緑・赤の3色に反応する複数のコーンオプシンをもち、詳細な映像を脳に伝達する。

一方、近年発見されたメラノプシンを含む節細胞は、形・色といった映像を脳に伝達することはできない。しかし興味深いことに、明るい暗いといった周囲の明暗情報を伝達できる。映像情報を伝達できないメラノプシンだが、ここ数年の「生物時計」の研究で、私たちの日常生活における睡眠・覚醒サイクルの形成に重要であることが明らかになってきた。

節細胞が捉えた光信号は、視神経を介し、視交叉上核に到達する。視交叉上核は私たちの頭のほぼ中心に位

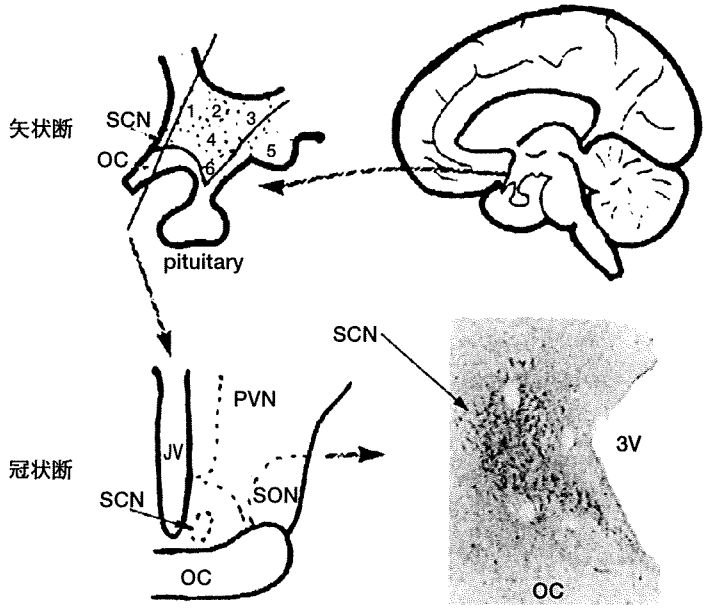

図2　ヒトの生物時計（視交叉上核）
SCN（suprachiasmatic nucleus）：視交叉上核、OC（optic chiasm）：視交叉、Pituitary：下垂体、PVN（periventricular nucleus）：室傍核、SON（supraoptic nucleus）：視索上核、3V（the third ventricle）：第3脳室。文献3より引用改変。

置し、「生物時計」ともよばれ、睡眠覚醒を調節すると同時に、各臓器に昼・夜の24時間周期の情報を神経連絡・ホルモンを介して伝達する。また、メラノプシンを含む節細胞は、外側膝状体にも連絡している。外側膝状体は桿体・錐体細胞からの映像を受け取る大脳皮質視覚野への中継地点である。このことは、節細胞から入力された明暗情報が桿体・錐体細胞の映像情報を修飾する可能性も示している（図2）。

赤ちゃんは外の世界をどのように見ているのか？

赤ちゃんはいつから光を感じ、外の世界をどのように見ているのだろうか？

この疑問に対する答えは、桿体細胞（ロドプシン）・錐体細胞（コーンオプシン）・節細胞（メラノプシン）の3つの光センサーの発達過程を考えることがヒントになる。

1. 節細胞は妊娠30週前後から働き始める

実は節細胞は、視蓋前域オリーブ核（olivary pretectal nuclei）にも神経を伸ばし、光が誘導する瞳孔の縮瞳（瞳孔反射）を調節している。つまり、瞳孔反射によって節細胞が機能しているかどうかを簡単に確かめることができる。ヒト早産児においては、瞳孔反射が妊娠30週から確認できることから、節細胞（メラノプシン）は少なくとも妊娠30週から機能していることがわかる。また、視覚のしくみがヒトに近いマントヒヒ胎児を調べた研究では、さらに早くヒト早産児が光に反応する可能性が示された[4]。この研究では、妊娠25～28週のヒト早産児に相当するマントヒヒ胎児を帝王切開にて出生させ、夜間5,000ルクスの光を当て胎児の生物時計「視交叉上核」の反応を確かめた。この実験では、光刺激で視交叉上核における神経活動が高まるとc-fos遺伝子の発現が上昇することを利用している（c-fos遺伝子は元々、がん・成長分化にかかわる遺伝子の発現を調節するが、脳科学ではニューロン活動性の指標としても頻繁に用いられる）。実際、この光照射によって視交叉上核のc-fos遺伝子の発現が上昇し、妊娠25～28週のヒト胎児においても節細胞から脳へ光信号が伝達されると推測された。

2. 桿体・錐体細胞は生後働き始める

一方、映像情報をキャッチする桿体・錐体細胞の発達は心理・電気生理の2つのアプローチから確かめられている[5]。

心理学では、「選考注視法」という技法で新生児が顔の表情・色を見分ける時期を明らかにしている。「選考注視法」では、赤ちゃんが新しく提示された対象物をより長い時間注視するという特徴を利用して、対象物が同じものか、新しいものかを赤ちゃ

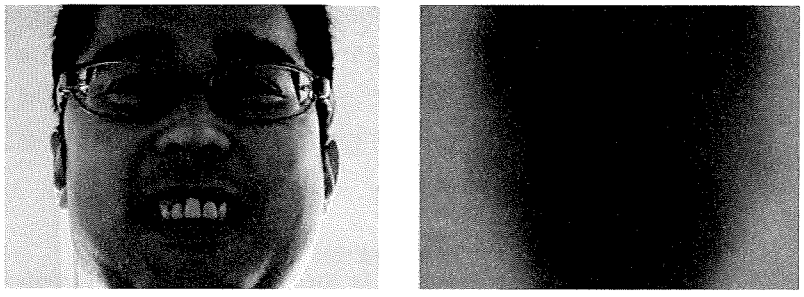

図3　早産児が下から見上げた大人の顔
a：大人の目から見たモデルの顔（実際はカラー写真）、b：妊娠35週相当の早産児から見た同じ顔のイメージ図（白黒写真）（向田茂氏（北海道情報大学情報メディア学部）作成）。

んが区別するのを確かめる。フィールドは、生後36時間の新生児に対し、女性の顔をモデルとした幸福・悲しみ・驚きの各表情刺激を提示した。

この実験では、表情刺激のタイプが変化した時点で、新しい表情刺激に対する新生児の注視時間が増加し、新生児が表情の違いを識別することが明らかになった。同様に選考注視法で赤ちゃんの色知覚（＝錐体細胞の機能）の検討したところ、緑・赤の区別ができるのは生後2か月頃からで、新生児は色の識別が不完全なため白黒の世界に住んでいることがわかった。これらの研究から、形の識別にかかわる桿体細胞は生後36時間以降、色・形の識別にかかわる錐体細胞は生後2か月以降に働き始めると推測されている。

心理学の結果は、電気生理学的アプローチとも一致している。光刺激に対する網膜電位（Electroretinogram：ERG）は、おもに桿体・錐体細胞が存在する網膜外側の組織に由来している。このERGを各発達段階で確かめたところ、妊娠36週の早産児で観察されるERGの反応は大人の10分の1以下で、早産児では桿体・錐体細胞が十分に機能していない結果となった。発達が進み生後6か月になると、大人と同程度のERGの反応が認められるようになってくる。

これら3つの光センサーの情報から、妊娠35週の赤ちゃんが見る世界をシミュレーションすると図3のようになる。早産で生まれた赤ちゃんは、形といった映像の情報よりも、明暗の光情報をおもに取り込んでいるので、ぼんやりとした白黒テレビの世界を見ていると想像される。

3. ノックアウトマウスにおける光受容体の発達研究

節細胞が桿体・錐体細胞より早く発達することを直接証明した研究がある。それは、ヒトとは動物種が異なるが、メラノプシン遺伝子を取り除いたマウス（ノックアウトマウス）の研究である。

先述のマントヒヒ胎児と同じように、マウス新生児に光刺激を与えると、生後0日より視交叉上核（生物時計）のc-fos遺伝子の発現が上昇し、マウスでは出生日より光情報が脳（視交叉上核）に到達することが明らかになった。ところが、メラノプシン遺伝子を取り除いたノックアウトマウスでは、光刺激に対する視交叉上核のc-fos遺伝子の反応は生後しばらく確認できず、反応がようやく確認できたのは生後14日目だった[7]。つまり、通常のマウスでは、生後2週間は桿体・錐体細胞が機能せず、その期間、節細胞のみが光の脳への伝達を行っていたことになる。この結果から、マウスにおいては生後2週間まで脳への光伝達はメラノプシンのみが、生後2週間以降は、ロドプシン・コーンオプシンが加わり処理が行われると推測された。

光環境は早産児の発達にどのように影響するのか？

以上の研究より、早産児は少なくともメラノプシンを使い、明暗情報を脳で処理していると考えてよさそうだ。早産児の発達と光環境について興味深い観察結果がある。これらの研究によると、昼夜の区別のある光環境（明暗環境）で保育された早産児は、24時間明るい環境（恒明環境）あるいは、反対に1日中暗い環境（恒暗環境）で保育された早産児より体重増加がよかった[8]。どうして明暗環境で育った早産児の体重増加がよいのか、そのメカニズムはまだよくわかっていない。

ただ、マウスを対象とした研究から、少なくともずっと明るい恒明環境が赤ちゃんの生物時計を細胞レベルで乱すことは明らかになった。赤ちゃんにかぎらず、大人のマウスも恒明環境に長期間さらされると生物時計がうまく機能しなくなる。生物時計の本体は、数万個の神経細胞が集合した視交叉上核とよばれる神経細胞の固まりからできていて、頭のほぼ中央に2つ並び、1組のペアとして存在する（図4）。この個々の神経細胞は独立してサーカディアン・リズムをもち、明暗環境では24時間周期で活動レベルの上昇・下降を一斉に繰り返している。ところが、恒明環境では、この神経細胞間の協調が崩れ、個々の神経細胞がバラバラのタイミングで活動し始める。この視交叉上核の活動の様子は、遺伝子工学を利用すると、生物時計を動かす遺伝子「時計遺伝子」をマーカーにして観察することができる。我々は、時計遺伝子Period1（ピリオド・ワン）のスイッチ（プロモーター）にクラゲ発光タンパク（Green Florescent Protein：GFP）の遺伝子を組込み、視交叉上核のPeriod1の活動をGFPの光で観察した。赤ちゃんマウス、大人のマウスにおける視交叉上核の神経活動の様子は、米国ヴァンダービルト大学のWebサイト内（赤ちゃんマウス：http://www.cas.vanderbilt.edu/PediatrRes2005OhtaMitchellMcMahon、大人のマウス：http://vvrc.vanderbilt.edu/NatNeurosci2005OhtaYamazakiMcMahon）で、それぞれ約3日間の記録を早送

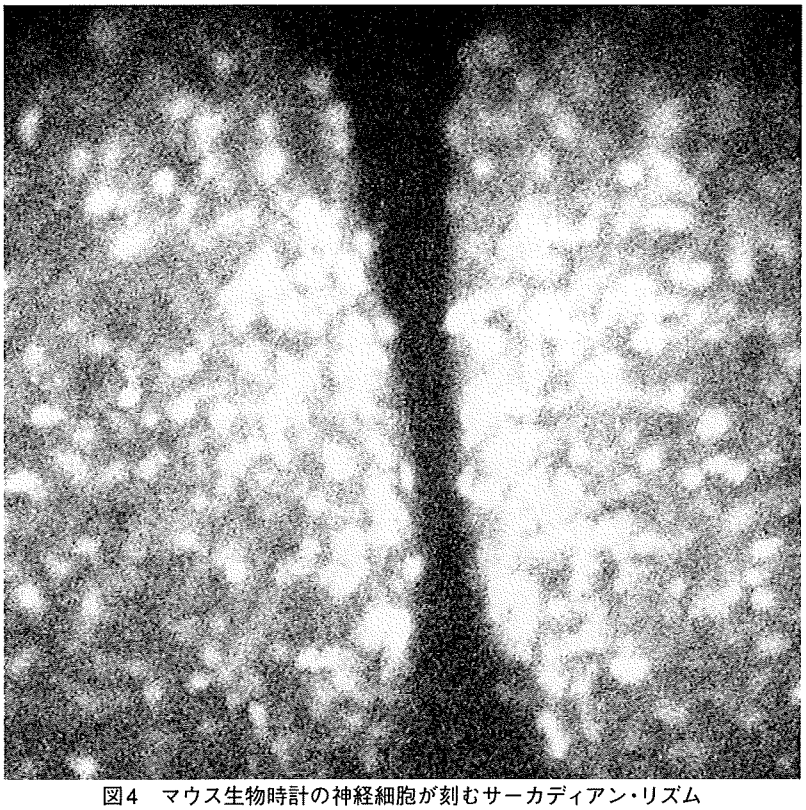

図4　マウス生物時計の神経細胞が刻むサーカディアン・リズム
生物時計（左右2つの視交叉上核）に存在する個々の神経細胞が24時間周期で発光を繰り返す。神経細胞内の生物時計の遺伝子Period1の働きに合わせ、遺伝子操作によって組み込まれたクラゲ発光タンパクが発色する（写真の白く光る部分。実際は緑色。1つ1つの粒が個々の神経細胞に対応している）。

りの動画として見ることができる。

また、恒明環境で飼育されたマウスでは、睡眠覚醒サイクルも24時間周期ではなく1～3時間周期となり、1日に複数の睡眠・覚醒サイクルを頻繁に繰り返す。このような睡眠サイクルの乱れが、睡眠によって誘導される成長ホルモンなどの成長促進物質の分泌を乱している可能性がある。また、赤ちゃんマウスを対象とした研究から、大人の生物時計より赤ちゃんの生物時計の方が光環境により敏感であり、恒明環境で乱れた赤ちゃんの生物時計は、規則正しい明暗サイクルで効果的に矯正できることが明らかになった。[9,10]。

おわりに―赤ちゃんの生体光回路を制御して―

従来から、心理学的アプローチを中心に赤ちゃんの視覚発達の研究が多数行われてきた。しかし、その生理学的・分子生物学的メカニズムはまだよくわかっていない。触覚・嗅覚・聴覚・味覚に比べ、赤ちゃんの視覚は出生時に未熟なため、生後環境の影響を最も受けやすい知覚機能といえる。とくに、保育器という人工環境で平均3か月間も生活する早産児にとって、視覚環境の整備の意義は大きい。過去の臨床研究で指摘されたように、光環境は赤ちゃんの体重増加といった身体発達に影響する。これまでの知見では、節細胞（メラノプシン）を光センサーとする生物時計は、ホルモン・神経伝達を介し、光情報を体全体の器官に伝達できる唯一のシステムである。この体全体に張り巡らされた生体光回路「生物時計」の詳細を明らかにし、最適な視覚環境を見つけることが赤ちゃんのより健全な発達を実現する1つの鍵になるだろう。将来、異なる波長の光を使い生体光回路のさまざまなスイッチを押すことにより、代謝・内分泌の視点から早産児のより適切な発育を実現することできるかもしれない。

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神奈川県立こども医療センター小児内科での勤務を経て、北海道大学大学院医学研究科博士課程修了。米国ヴァンダービルト大学生物科学部Research associate、東北大学病院周産母子センター医員、助手を経て、2007年より現職。専門は、発達生理学、小児科学、時間生物学。生物時計の発達、環境センサー遺伝子、人工環境デザイン、次世代人工保育器の開発研究に従事。早産児・新生児の保育環境デザインについての研究成果を挙げている。2004年、Butterworth dissertation Award（国際乳幼児学会）、2005年、日本時間生物学会学会奨励賞を受賞。著書に、「きちんとわかる時計遺伝子」（分担、白日社、2007年）。

BASIC SCIENCE: OBSTETRICS

Effect of intrauterine inflammation on fetal cerebral hemodynamics and white-matter injury in chronically instrumented fetal sheep

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OBJECTIVE: The purpose of this study was to analyze the effects of intrauterine inflammation on cerebral hemodynamics and white-matter injury in premature fetal sheep.

STUDY DESIGN: Fetuses were given an intravenous infusion of granulocyte colony-stimulating factor and an intraamniotic infusion of endotoxin; the fetuses were then assigned randomly to an acute hemorrhage group, an exchange transfusion group, or a control group. During each insult, the cerebral hemodynamics were assessed with near-infrared spectroscopy. Finally, the fetuses were processed for neuropathologic analysis and compared statistically.

RESULTS: Necrotizing funisitis and chorioamnionitis were induced in all the fetuses. A significant decrease in the blood oxygen content and an increase in the brain total hemoglobin level were observed after the endotoxin infusion. Soon after hemodynamic insult, the fetuses in both the acute hemorrhage and the exchange transfusion groups showed an abrupt decrease in the total brain hemoglobin level; 4 of the 5 fetuses in each treatment group, but none of the fetuses in the control group, exhibited periventricular leukomalacia.

CONCLUSION: Hemorrhagic hypotension or anemic hypoxemia might induce a sudden cessation of fetal brain-sparing effects through progressive inflammatory hypoxemia, which results in focal white-matter injuries.

Key words: cerebral white-matter injury, chorioamnionitis, fetal sheep, funisitis, periventricular leukomalacia

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Cerebral white-matter injuries (WMIs), such as periventricular leukomalacia (PVL), are a major cause of long-term neurologic disabilities in premature infants and are associated closely with the subsequent development of cerebral palsy and cognitive deficits later in childhood.[1-3] Although the pathogenesis of WMIs and possible measures for the prevention of these injuries have not yet been clarified fully, recent clinical and experimental studies have shown that in utero damage to immature oligodendrocytes as a result of infectious inflammation, such as chorioamnionitis or funisitis, is a key prenatal factor that contributes to the development of WMIs.[4-7]

These aforementioned investigations suggest that the in utero release of neurotoxic factors (such as inflammatory cytokines) from regions of inflammation appears to exert direct effects that damage the vulnerable oligodendrocyte system, which results in diffuse myelination disturbance (diffuse WMI) in premature fetuses. On the other hand, because expanded placental vasculitis might disturb the villous gas exchange, advanced intrauterine inflammation might also cause ischemic hypoxia in deep white matter, which results in focal necrotic lesions (focal WMI [ie, PVL]). Thus, septic inflammatory responses might affect cerebral blood flow and systemic hemodynamics; however, only a few reports have championed this viewpoint.[8-10]

The aim of the present study was to analyze the effects of intrauterine inflammation on cerebral hemodynamics and focal WMI in chronically instrumented fetal sheep. In previous studies,[11-13] we experimentally induced antenatal PVL using hemorrhagic hypotension (but not hypoxia) in chronically instrumented fetal sheep and clarified the characteristics of fetal cerebral hemodynamics during the induction of PVL using a continuous, noninvasive near-infrared spectroscopy (NIRS) technique. Additionally, we developed an an-

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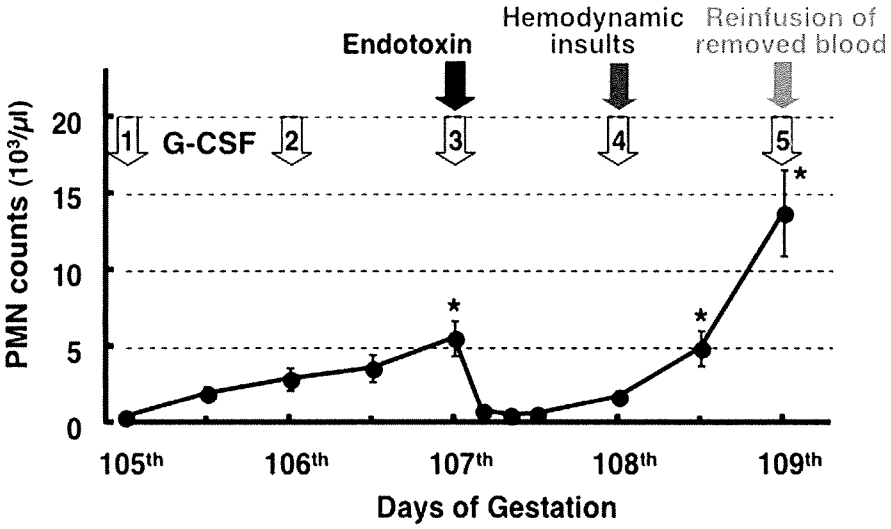
FIGURE 1

Changes in the PMNL counts of fetal abdominal aortic blood over time



All fetuses (n = 15) were intravenously infused with 40 μg of G-CSF daily from days 105-109 of gestation and were infused with 20 mg of endotoxin into the amniotic cavity once on day 107 of gestation. The *numbers within the open arrows* indicate the timing of the G-CSF infusion. At 24 hours after the endotoxin infusion on day 108 of gestation, the hemodynamic insults were performed in each of the 3 groups; the removed blood was then returned to the fetuses in the hemorrhage and exchange transfusion groups at 24 hours after the insults on day 109 of gestation. All data are expressed as the mean ± SEM. The *asterisk* indicates $P < .05$ (Dunnett test), compared with the value on day 105 of gestation.

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imal model of necrotizing funisitis and chorioamnionitis (severe in utero inflammations of the umbilical cord and fetal membrane, respectively) using the intravenous administration of granulocyte colony-stimulating factor (G-CSF) and the intraamniotic administration of endotoxin in premature fetal sheep.[14,15] By combining these previously developed inflammatory and hemodynamic models in the present study, we attempted to examine experimentally whether preexisting intrauterine inflammation exacerbated fetal brain damage that is induced by hemodynamic insults.

Of note, the major purpose of the inflammatory model described earlier was to reproduce only the fetal side of the in utero inflammatory reactions, without inducing preterm labor. The G-CSF pretreatment was considered to be reasonable because premature infants with chorioamnionitis reportedly have significantly higher G-CSF levels in their umbilical cord blood than premature infants without chorioamnionitis.[16] Moreover, the results of previous experiments suggested that the dose of endotoxin that was administered intraamniotically in the present inflammatory model was too weak to induce WMIs.[7,8] Therefore, to exacerbate the in utero inflammation in the present study, we intravenously administered G-CSF in addition to administering endotoxin into the amniotic cavity.[14]

Materials and Methods

Animal preparation

This study was approved by the Animal Care and Use Committee of Tohoku University Graduate School of Medicine, Sendai, Japan (No. 15-128) and was performed between November 2004 and March 2006. The preparations and protocol that were used in the present experiment were largely the same as those used in our previous studies.[11,13-15] Briefly, a total of 15 Suffolk ewes with timed pregnancies underwent surgery on days 102-103 of gestation. The ewes were intubated, ventilated, and anesthetized with 1.5-2% isoflurane during all the procedures. After a laparotomy and hysterotomy, 3 electrodes were fixed to the fetal chest wall, and polyvinyl catheters were inserted into the fetal superior vena cava, inferior vena cava, distal abdominal aorta, and amniotic cavity. NIRS optodes were applied directly to the fetal skull symmetrically on each side of the central suture.[11] All electrodes, catheters, and optodes were exteriorized through a small incision in the flank of each ewe. After surgery, the ewes were unrestrained and housed in individual cages, with free access to water and food throughout the study period. A recovery period of at least 2 days was allowed before the experiments were started. During this period, the recovery of the fetal physiologic parameters to their baseline values was confirmed and, antibiotics were administered additionally to the mother, fetus, and amniotic cavity based on the results of Gram stain tests with the use of amniotic fluid smears.

Physiologic parameter measurements

Fetal heart rate and arterial and amniotic pressure were monitored continuously with a polygraph and were recorded on a personal computer throughout the study. All fetal arterial pressure values were corrected for the amniotic fluid pressure. The total leukocyte counts (Celltac MEK-5254; Nihon Kohden Co, Tokyo, Japan), hemoglobin levels, oxygen content (OSM3 hemoximeter; Radiometer Medical, A/S, Copenhagen, Denmark), pH, base excess, P_{CO_2} and P_{O_2} levels, and lactate level (Blood Gas System 860; Bayer Medical Co, Sudbury, United Kingdom) were measured in blood samples (0.5 mL) that were taken from the fetal abdominal aorta every 12 hours throughout the experiment. Polymorphonuclear leukocytes (PMNL) were identified with differential staining (May-Giemsa) and were counted microscopically in relation to the total leukocyte count. The blood gas data were corrected with the use of the maternal rectal temperature.

NIRS

NIRS of the brain was performed with a hemoximeter (OM-100A; Shimazu, Kyoto, Japan).[11] The fetal skull was illuminated with a halogen light with the use of a NIRS optode; the light that was transmitted through the cranial bone and the cerebral tissue was then measured by another optode. Attenuation measurements for 4 wavelengths (700, 730, 750, and 805 nm) were transformed into relative changes in chromophore concentrations at 1-second intervals, according to a previously described algorithm[17]; this procedure rendered the relative changes in oxyhemoglobin (oxy-Hb), deoxyhemoglobin (deoxy-Hb), and total hemoglobin (oxy-Hb + deoxy-Hb) concentrations. All NIRS parameters were expressed as a percentile change from the baseline value (averaged from the values that were recorded for 3 hours before the endotoxin infusion) and were stored on a personal computer.

Estimation of fetoplacental blood volume

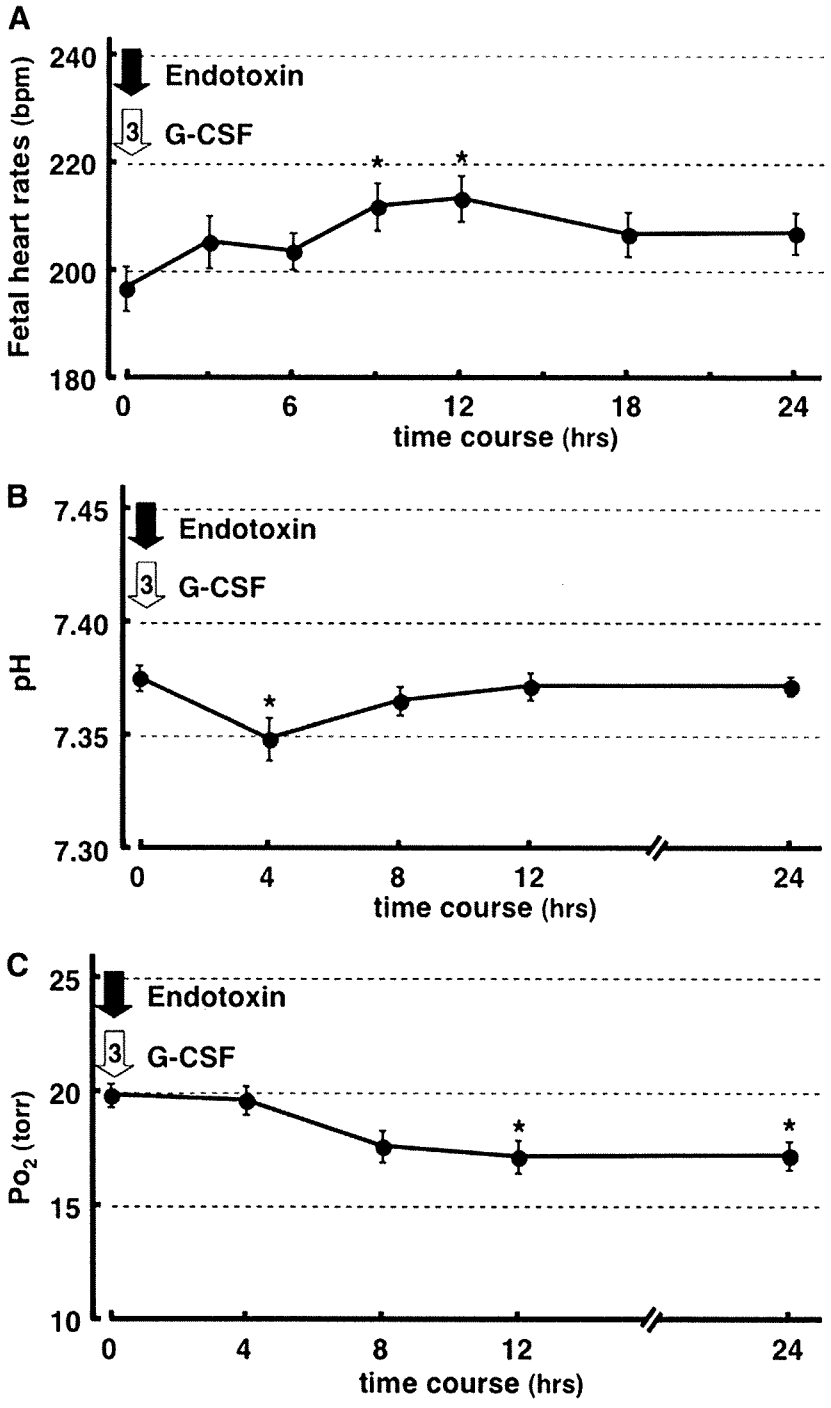
At least 48 hours after the operation on days 104-105 of gestation, an isovolumic exchange transfusion was performed for each fetus with 20 mL of heparinized fresh plasma from another fetal sheep. The hematocrit values were measured before and after the exchange transfusion. The actual fetoplacental blood volume was calculated with the following formula: V = v/ln (hematocrit before transfusion/hematocrit after transfusion), where V represents the fetoplacental blood volume and v represents the volume of exchanged blood.[11-13] Two hours after the exchange transfusion, the erythrocytes that had been separated from the removed blood were returned to the fetus over a 5-hour period.

Experimental protocol

After the fetoplacental blood volume had been estimated, the fetuses (n = 15) were infused with 40 μg of G-CSF (Neutrogin; Chugai Co Ltd, Tokyo, Japan) solublized in 2 mL of saline solution that was administered into the inferior vena cava daily from days 105-109 of gestation to increase the PMNL counts in the circulating blood (Figure 1).[14] In addition, the

FIGURE 2

Changes in physiologic parameters over time for 24 hours after endotoxin infusion



A, Heart rates, **B**, pH, **C**, Po_2, **D**, oxygen content (*closed circles*) and lactate level (*open squares*), and **E**, hemoglobin level of fetal abdominal aortic blood; **F**, oxy-Hb, deoxy-Hb, and total hemoglobin levels in fetal cerebral tissue, as measured with NIRS (*closed squares*, oxy-Hb; *open squares*, deoxy-Hb; *closed circles*, total hemoglobin). The *numbers within the open arrows* indicate the timing of the G-CSF infusion. All data are expressed as the mean ± SEM. The *asterisks* indicate $P < .05$ (Dunnett test), compared with the values just before endotoxin infusion.

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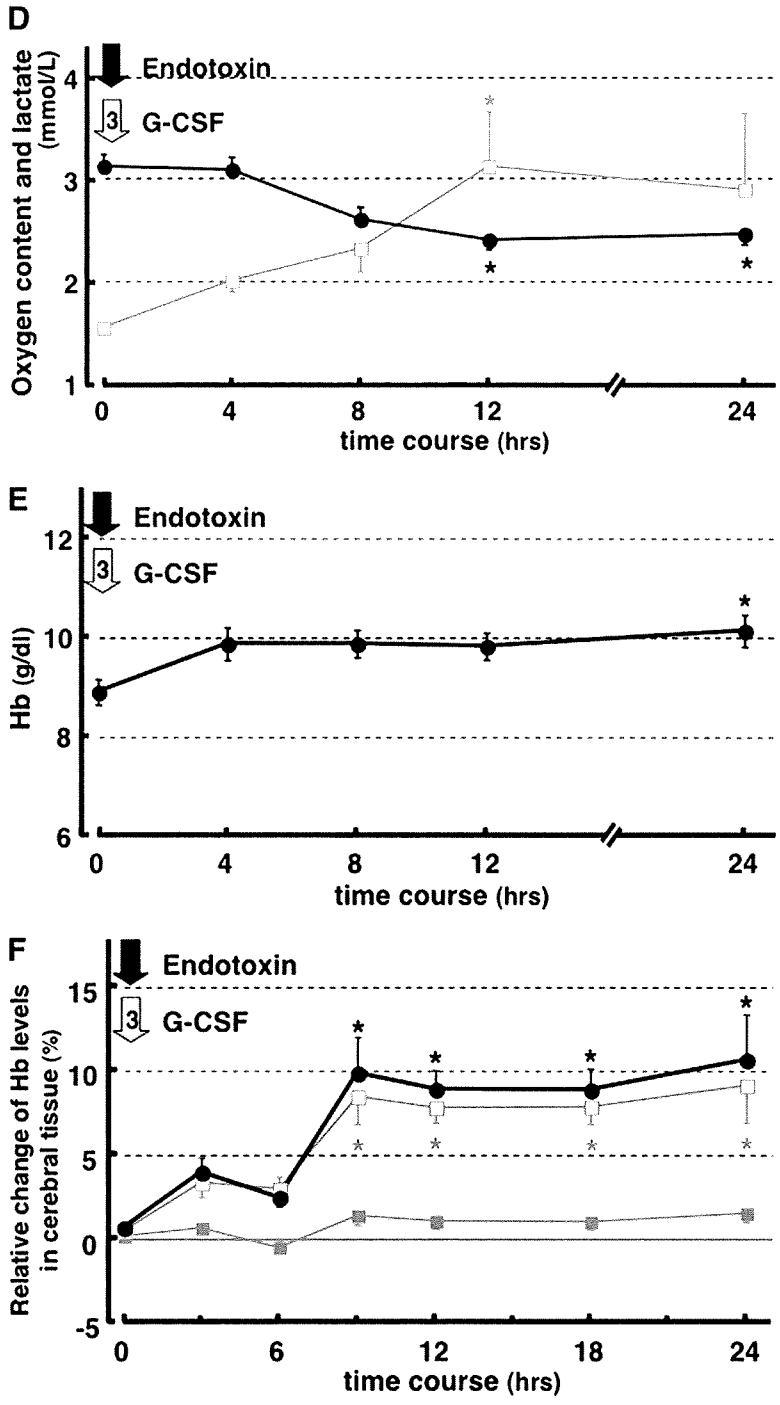
FIGURE 2

Changes in physiologic parameters over time for 24 hours after endotoxin infusion *(continued)*



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fetuses were injected intraamniotically with 20 mg of endotoxin (*Escherichia coli* 055:B5 endotoxin; Sigma Chemical Co, St Louis, MO) solublized in 5 mL of saline solution once on day 107 of gestation to activate the PMNLs and to induce inflammation around the amniotic cavity (Figure 1).[18]

Twenty-four hours after the endotoxin infusion on day 108 of gestation, the fetuses were divided randomly into 3 groups (n = 5 each): a hemorrhage group, an exchange transfusion group, and a control group. The following experiments were then conducted.[11-13] To induce systemic fetal hypotension in the hemorrhage group, approximately 35-40% of the fetoplacental blood volume was withdrawn at a constant rate from the inferior vena cava catheter over a period of 20 minutes; 24 hours after removal, the blood was returned to the fetuses over a period of 5 hours (Figure 1). In the exchange transfusion group, an exchange transfusion of approximately 35-40% of the fetoplacental blood volume was performed with heparinized fresh plasma to create a level of anemia that was equivalent to that in the hemorrhage group without inducing hypotension; the erythrocytes that were separated from the removed fetal blood were adjusted to the same volume as the exchange volume with the use of sodium chloride solution and then were returned to the fetuses over a period of 5 hours beginning 24 hours after the exchange transfusion (Figure 1). In the control group, no hemodynamic insult was performed.

The fetal heart rate, mean blood pressure, and NIRS parameters at each time point represent the averages of data that were obtained every 5 minutes. To obtain the PMNL counts, hemoglobin and lactate levels, oxygen content, and blood gas data, an abdominal aortic blood sample (0.5 mL) was taken just before the endotoxin infusion at 4, 8, 12, and 24 hours after the endotoxin infusion, just before the hemodynamic insults, and at 20 and 60 minutes and 2, 4, 6, 12, 18, and 24 hours after the hemodynamic insults. The values of the PMNL counts for every 12 hours from days 105-109 of gestation were added to evaluate changes over time throughout the study period. After each sampling, an equivalent volume of heparinized and stored maternal blood was infused into the fetus through the venous catheter.

Histopathologic examination

Six days after the endotoxin infusion, cesarean sections were performed, and the fetuses were weighed. The fetal membrane, umbilical cord, and placentomas were placed in 10% formalin solution for

TABLE 1
Comparison of basic fetal characteristics

Variable	Group: Hemorrhage, (n = 5)	Exchange transfusion, (n = 5)	Control, (n = 5)
Fetoplacental blood volume (mL)	250 ± 33	201 ± 16	231 ± 11
Withdrawn blood volume (%)	38.5 ± 1.2	38.4 ± 0.6	
Brain weight (g)	32.1 ± 1.7	33.3 ± 1.0	31.2 ± 0.5
Bodyweight (kg)	1.94 ± 0.12	1.92 ± 0.11	2.04 ± 0.13
Brain/bodyweight ratio (%)	1.66 ± 0.05	1.75 ± 0.08	1.56 ± 0.12

All variables are expressed as the mean ± SEM. The body and brain weights were measured 6 days after the hemodynamic insult.
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fixation. Under anesthesia, the fetal brains were perfused with 10% neutralized buffered formalin for fixation, removed, and weighed. The cerebral hemispheres were cut into 4 standardized coronal sections at the level of the frontal lobe, the anterior basal ganglia, the mamillary bodies, and the occipital lobe. Multiple additional sections of the cerebellum, midbrain, pons, and medulla oblongata were also obtained. After each section was observed macroscopically, a histopathologic evaluation was performed with 4-μm sections that were stained with hematoxylin and eosin.

With the criteria proposed by Banker and Larroche,[1] *PVL* was defined as the presence of scattered round neuroaxonal swelling and focal coagulation necrosis with infiltration by microglia/macrophages that is localized within the deep white matter around the lateral ventricles. The existence and degree of PVL were evaluated by scoring the existence of PVL (0 = PVL negative; 1 = PVL positive) and by counting the number of necrotic foci (> 500 μm in diameter) within the periventricular white matter, respectively. *Necrotizing funisitis* was defined by the criteria of Navarro and Blanc.[19,20] The same observer (Y.K.) made all the histopathologic assessments in a blinded fashion.

TABLE 2
Comparison of values of physiologic factors just before the hemodynamic insults

Variable	Group: Hemorrhage, (n = 5)	Exchange transfusion, (n = 5)	Control, (n = 5)
PMNL count ($10^3/\mu$L)	2.6 ± 1.0	1.5 ± 0.4	1.1 ± 0.4
Fetal heart rate (beat/min)	203 ± 8	210 ± 9	208 ± 2
Mean blood pressure (mm Hg)	39.2 ± 1.6	39.4 ± 1.7	41.1 ± 1.8
pH	7.36 ± 0.01	7.37 ± 0.00	7.38 ± 0.01
Pco_2 (torr)	56.1 ± 1.9	56.6 ± 0.4	56.2 ± 1.0
Po_2 (torr)	16.7 ± 0.8	18.3 ± 1.7	16.7 ± 0.4
Hemoglobin level (g/dL)	10.7 ± 0.7	9.6 ± 0.5	10.1 ± 0.4
Oxygen content (mmol/L)	2.6 ± 03	2.5 ± 0.3	2.3 ± 0.1
Lactate (mmol/L)	4.3 ± 2.2	2.1 ± 0.5	2.2 ± 0.2
Total hemoglobin (%)	11.3 ± 2.8	9.7 ± 3.9	7.3 ± 2.7
Oxy-Hb (%)	2.1 ± 0.5	1.9 ± 0.9	0.6 ± 0.8
Deoxy-Hb (%)	9.2 ± 2.4	7.8 ± 3.1	6.7 ± 2.2

Deoxy-Hb, deoxyhemoglobin; *Oxy-Hb*, oxyhemoglobin; *PMNL*, polymorphonuclear leukocytes
All variables are expressed as the mean ± SEM.
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Statistical analysis

All values were expressed as the mean ± SEM. The Dunnett test was performed to test for significant changes in the physiologic parameters from the baseline values during the 24 hours after the endotoxin infusion. For the next 24-hour period, a repeated-measures analysis of variance was performed to compare the changes in the physiologic parameters over time between the control group and the hemorrhage or exchange transfusion group; if significant changes were suggested over time, the Dunnett test was performed to test for significant changes from the values just before the hemodynamic insults in each group. Differences in continuous variables among the 3 groups were assessed with the 2-way Kruskal-Wallis test; if a significant difference was suggested, the Scheffe test was performed for multiple comparisons among the groups. Differences between 2 groups were assessed with the Wilcoxon signed-rank test for continuous variables. All probability values were 2-tailed, and a probability value of $< .05$ was considered significant.

RESULTS

Continuous monitoring of all parameters showed that all 15 fetuses were in good condition, with no occasions when the mean arterial pressure was < 35 mm Hg or the heart rate was < 100 beats/min during any 3-minute period in the study, except for immediately after the hemodynamic insults. A fetal pH of < 7.25 and a PO_2 of < 12 mm Hg did not occur at any time during the experiments. No intrauterine infections or signs of premature labor episodes occurred. All fetuses were available for physiologic and neuropathologic assessment.

TABLE 3
Comparison of neuropathologic findings in WMI

Variable	Group: Hemorrhage, (n = 5)	Exchange transfusion, (n = 5)	Control, (n = 5)
PVL (n)[a,b]	4	4	0
PVL score[a,c]	0.8 ± 0.2[d]	0.8 ± 0.2[d]	0.0 ± 0.0
PVL foci[a]	3.0 ± 1.8	3.0 ± 1.4	0.0 ± 0.0
Subcortical multifocal WMI (n)[b]	1	2	0

PVL, periventricular leukomalacia; *WMI*, white-matter injury.

[a] $P < .05$, comparing the values among the 3 groups (Kruskal-Wallis test); [b] Number of positive fetuses; [c] Expressed as the mean ± SEM; [d] $P < .05$, compared with the values of the control group (Scheffe test).

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G-CSF and endotoxin administration

Figure 1 shows the changes in the blood PMNL counts during G-CSF administration. Significant increases in the blood PMNL counts, compared with the baseline value ($0.4 \pm 0.8 \times 10^3/\mu L$), were found on day 107 ($5.6 \pm 1.1 \times 10^3/\mu L$), day 108 ($4.9 \pm 1.1 \times 10^3/\mu L$), and day 109 ($13.7 \pm 2.8 \times 10^3/\mu L$) of gestation ($P < .05$), whereas the blood PMNL count transiently decreased for 24 hours after the endotoxin infusion.

Significant changes in the heart rate, pH, Po_2, oxygen content, and lactate and hemoglobin levels were observed over time during the 24-hour period after endotoxin infusion ($P < .05$), but no changes were seen in any of the other parameters, including the mean blood pressure, Pco_2, and base excess in the fetal abdominal aorta. The fetal heart rates significantly increased from 9-12 hours after the endotoxin infusion, compared with the baseline value (Figure 2, A). Blood pH decreased significantly, but transiently, at 4 hours after the endotoxin infusion (Figure 2, B). The blood Po_2 and oxygen contents gradually decreased and then showed a significant decrease at 12 hours onward after the endotoxin infusion (Figure 2, C and D). The increases in the lactate level peaked at 12 hours after endotoxin infusion (Figure 2, D). The hemoglobin levels gradually increased and then significantly increased at 24 hours after the endotoxin infusion (Figure 2, E).

Figure 2, F shows the changes in the NIRS parameters for 24 hours after the endotoxin infusion. Significant changes over time were found with regard to the levels of deoxy-Hb and total hemoglobin, but not for oxy-Hb. Significant increases in both the total hemoglobin and deoxy-Hb levels, compared with the baseline values, were observed simultaneously from 9 hours onward after the endotoxin infusion and then continued for 15 hours ($P < .05$). However, the oxy-Hb levels showed no apparent changes, compared with the baseline value, during the same time period.

Hemodynamic insults

Table 1 shows the results of the statistical analysis of the basic fetal characteristics of the hemorrhage, exchange transfusion, and control groups. No significant differences among the 3 groups were found with regard to the estimated feto-placental blood volume, brain and bodyweight, or brain/bodyweight ratio, and no significant difference in the actual withdrawn blood volume (%) was noted between the hemorrhage and exchange transfusion groups. The values of the physiologic measures just before the hemodynamic insults are summarized and compared among the 3 groups in Table 2. No differences in the PMNL counts, fetal heat rate, mean blood pressure, pH, Pco_2, Po_2, hemoglobin level, oxygen content, lactate level, or NIRS parameters were observed among the 3 groups before the insults.

The parameters showed significant changes, compared with those in the control group, over the course of the hemodynamic insults and were identical in the hemorrhage and exchange transfusion groups (mean blood pressure [$P < .01$], blood hemoglobin level [$P < .01$] and oxygen content [$P < .01$] in the fetal abdominal aorta and total hemoglobin level [$P < .01$], oxy-Hb [$P < .01$], and deoxy-Hb [$P < .01$] in the fetal cerebral tissue.)

The mean blood pressure in the hemorrhage group decreased sharply during blood withdrawal, decreased significantly from 10-20 minutes after the start of the insult ($P < .05$), and then rapidly recovered within 30 minutes, whereas the mean blood pressure in the exchange transfusion group gradually increased and then recovered within the same period (Figure 3, A). The blood hemoglobin level dropped significantly from 6 hours onward after the insult in the hemorrhage group ($P < .05$) and 20 minutes onward after the insult in the exchange transfusion group ($P < .05$; Figure 3, B). Significant decreases in the oxygen content in the hemorrhage group were observed at 6, 18, and 24 hours after the insult ($P < .05$); those decreases in the exchange transfusion group occurred at 20 minutes and 18 and 24 hours after the insult ($P < .05$; Figure 3, C).

The changes in the NIRS parameters in the 3 groups over the course of the hemodynamic insults are shown in Figure 3D, E, and F. The oxy-Hb values in the hemorrhage and exchange transfusion groups slightly decreased soon after the insult and then gradually increased until the reinfusion of the removed blood; significant decreases from the values just before the insult were found only from 20 minutes to 6 hours after the insult in the hemorrhage group (Figure 3, D). The deoxy-Hb levels in the hemorrhage group showed a serious decrease from 30-60 minutes after the insult and then increased from 2 hours onward after the insult (Figure 3, E). The deoxy-Hb levels in the exchange transfusion group also decreased soon after the insult but did not deviate significantly from the value just before the insult; the only significant difference, compared

with the control group, was observed at 4 hours after the insult. The changes in the total hemoglobin level during the hemodynamic insult were quite similar to those for the deoxy-Hb level in each group (Figure 3, F). In the hemorrhage group, however, the significant reduction in the total hemoglobin level persisted from 20 minutes until 4 hours after the insult, at which time the systemic hypotension had already recovered (Figure 3, A). In the exchange transfusion group, on the other hand, the total hemoglobin levels were never less than the baseline value at any time point and did not change significantly from the value just before the insult, whereas a significant difference compared with the control group was found only at 4 hours after the insult. No significant difference in the magnitude of the maximal decrease in the total hemoglobin level between the hemorrhage (15.2% ± 2.8%) and the exchange transfusion group (10.4% ± 4.9%) was observed.

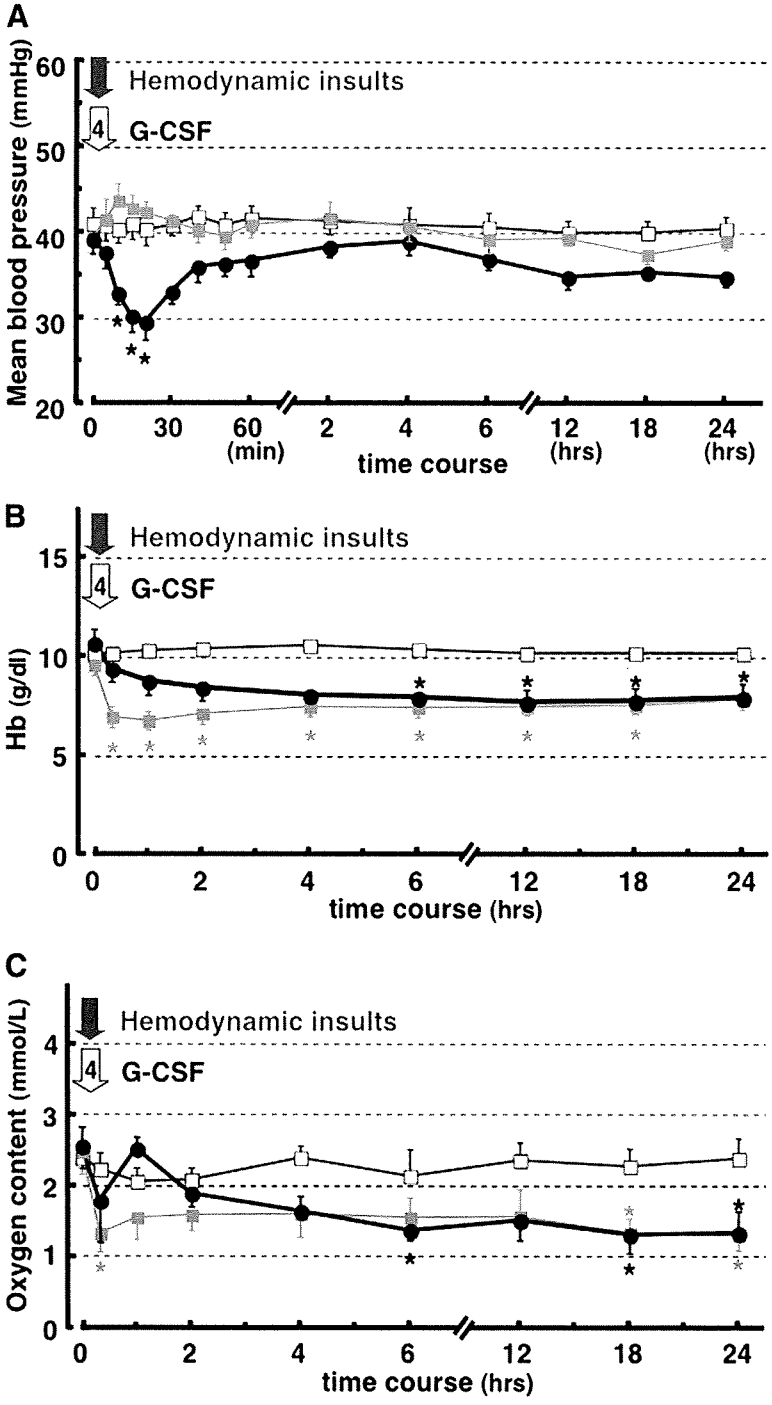
Histopathologic findings

In the histopathologic examination of the umbilical cord and chorioamnion, all 15 fetuses were diagnosed as having necrotizing funisitis and chorioamnionitis; numerous PMNLs had infiltrated from the vascular space toward the amniotic cavity, had accumulated and degenerated within the epithelial layers, then frequently had necrotized and peeled away, which was similar to our previously reported results.[14] In the placentomes, however, neither vascular PMNL infiltration nor degenerative changes were observed in the interdigitated villi of the fetal cotyledon.

The results of the neuropathologic examinations are summarized in Table 3. Four of the 5 fetuses in both the acute hemorrhage and exchange transfusion groups had PVL (Figure 4, A), but none of the fetuses in the control group had PVL. The PVL scores in both treatment groups were significantly higher than that in the control group ($P < .05$), although no significant difference in the number of PVL foci was observed between the acute hemorrhage (3.0 ± 1.8 pieces) and the exchange transfusion groups (3.0 ± 1.4 pieces). In addition to

FIGURE 3

Comparison of changes in physiologic parameters over the course of the hemodynamic insults



A, Mean blood pressure, **B,** hemoglobin level, **C,** lactate level, and **D,** oxygen content of fetal abdominal aortic blood; **E,** oxy-Hb, **F,** deoxy-Hb, and **G,** total hemoglobin levels in fetal cerebral tissue, as measured with NIRS (*closed circles*, hemorrhage group; *closed squares*, exchange transfusion group; *open squares*, control group). The *numbers within the open arrows* indicate the timing of the G-CSF infusion. All data are expressed as the mean ± SEM. The *asterisks* indicate $P < .05$ (Dunnett test), compared with the values just before endotoxin infusion. The *pound symbol* (#) indicates $P < .05$ (Scheffe test), compared with the values in the control group at each time point.

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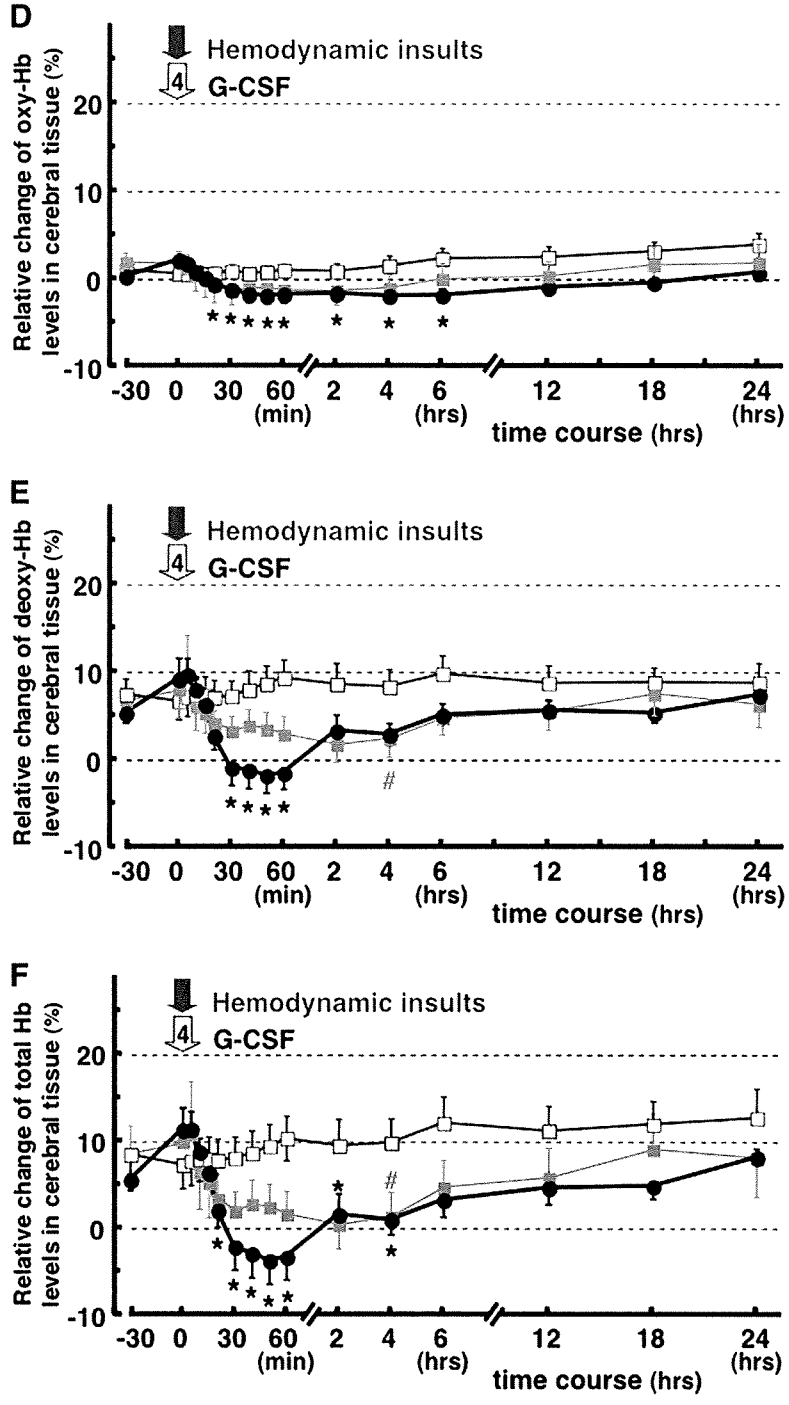
FIGURE 3

Comparison of changes in physiologic parameters over the course of the hemodynamic insults *(continued)*



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PVL, multiple petechial hemorrhages in the subcortical white matter (Figure 4, B) were found in 1 of the 4 fetuses in the acute hemorrhage group and in 2 of the 4 fetuses in the exchange transfusion group. These subcortical multifocal WMIs always surrounded a necrotic core lesion adjacent to a small vessel (Figure 4, C) within or close to an area where numerous leukocytes (which included PMNLs, monocytes and lymphocytes) were observed (Figure 4, D).

COMMENT

To induce exacerbated intrauterine inflammation in the premature fetal sheep that were used in this study, G-CSF first was administered intravenously to increase the circulating blood PMNL levels, and then endotoxin was infused into the amniotic cavity to activate the PMNLs in utero.[14] Consequently, all 15 fetuses exhibited both necrotizing funisitis and chorioamnionitis. The transient decrease in the blood PMNL counts just after endotoxin infusion (Figure 1) probably reflects the regional migration of massive numbers of PMNLs from the vascular space toward the amniotic cavity, which was reported previously in experiments on intraamniotic endotoxin infusion in pregnant sheep.[8,14,18]

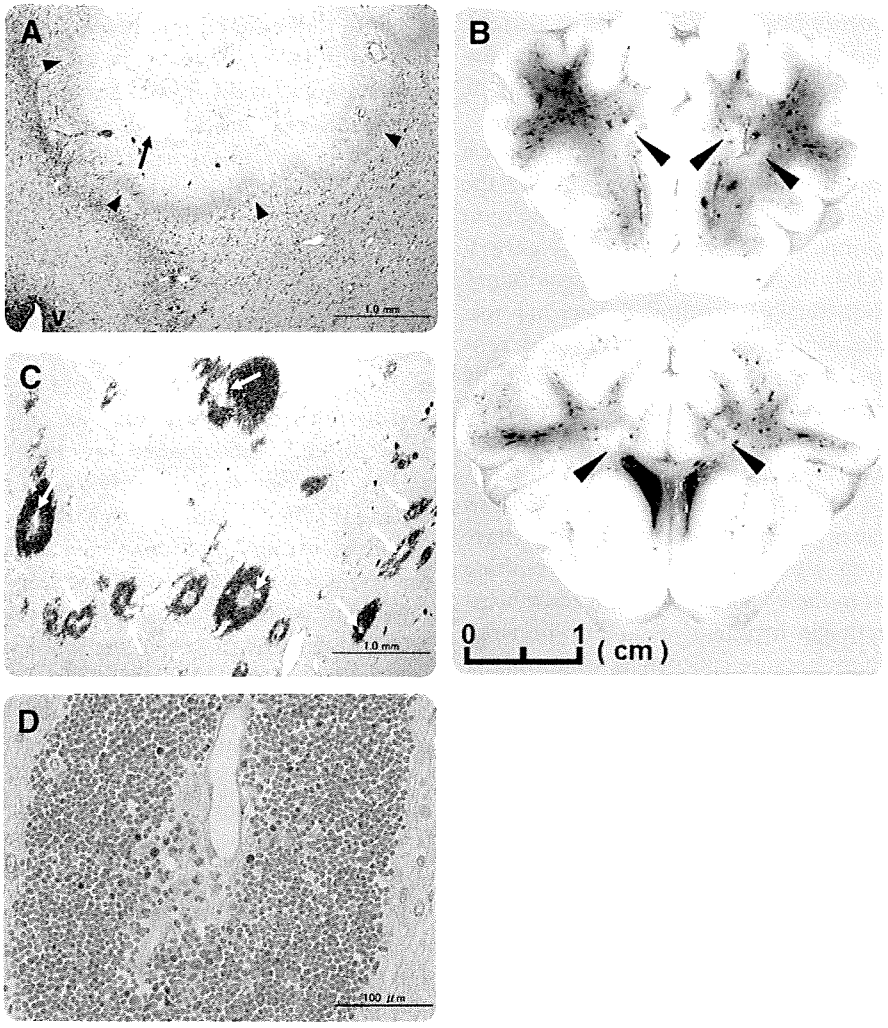
As expected, the data suggested that the regional inflammatory changes induced in utero caused systemic hypoxia and hypovolemia in the premature fetal sheep. The development of hypoxia is supported by the decreased blood oxygen content, Po_2, and pH and by the increased heart rate and blood lactate levels that occurred from 4-12 hours onward after endotoxin infusion (Figure 2, A-D). A possible explanation for this phenomenon is that the expanded vasculitis in the fetal membrane and umbilical cord may have caused functional or reversible changes (such as vasoconstriction or interstitial edema) that disturbed the villous gas exchange in the placentomes; however, no histopathologic changes in the villous tissue were observed.[6,9] The systemic hypovolemia can be explained by the gradual increase in blood hemoglobin levels (Figure 2, E), because the advanced vasculitis in utero may have injured the endothelium and increased the vascular permeability, which lead to the progressive loss of plasma moieties from the blood.[6,9]

NIRS measurements of the cerebral blood volume showed a compensatory increase in fetal cerebral blood flow in response to systemic changes that would have otherwise reduced cerebral oxygen delivery. After endotoxin infusion, the

oxy-Hb level in the cerebral tissue was maintained at its baseline value, even though the blood oxygen content significantly decreased during the same period; moreover, the brain deoxy-Hb and total hemoglobin levels significantly and continuously increased, compared with their baseline values (Figure 2, F). These changes may have arisen as a result of the increased cerebral blood flow that was induced by a compensatory redistribution of the systemic blood flow, or the so-called brain-sparing effect,[21,22] and the cerebral hemodynamic changes that were observed in an experiment that involved the intravenous administration of endotoxin in premature fetal sheep.[23]

In situations in which the brain-sparing effect was maintained in the fetal brain, 3 noticeable and interesting differences in the results for the hemodynamic insult groups (hemorrhagic hypotension and anemic hypoxemia) were observed, compared with our results that had been obtained in a previous study with the same protocol but without intrauterine inflammation.[13] First, the reduction in the total hemoglobin level in the fetal brain tissues differed between our current and previous studies (Figure 3, F), whereas no differences in systemic changes that were related to hemorrhagic hypotension or anemic hypoxemia were observed between our 2 studies (Figure 3, A-C). Specifically, the magnitudes of the total reduction in hemoglobin level just after insult in the hemorrhage (15.2% ± 2.8%) and exchange transfusion (10.4% ± 4.9%) groups in the current study were significantly greater than those in the hemorrhage (4.2% ± 0.8%) and exchange transfusion (3.3% ± 0.5%) groups in the previous study, respectively ($P < .05$; Wilcoxon signed-rank test), although no remarkable difference in the change in the total hemoglobin level was observed between the 2 studies. To our surprise, the discrepancy in the magnitudes of the total reduction in hemoglobin level between the 2 studies was equal to that of the compensatory increase in the fetal cerebral blood flow (Table 2), which had already been induced by the brain-sparing effect.

FIGURE 4
The neuropathologic findings of WMI



A, Hematoxylin and eosin-stained section of a periventricular focal cerebral white-matter injury (WMI) shows focal coagulation necrosis (*arrowheads*) in the deep white matter around the lateral ventricle (*v*), in which cavity formation (*arrow*) was noted. **B,** Macroscopic findings of fetal brain showing WMIs in coronal sections of the cerebral hemispheres at the levels of the frontal lobe (*top*) and the anterior basal ganglia (*bottom*). Multifocal petechial hemorrhages were observed within the subcortical white matter; periventricular focal coagulation necrosis was noted as small white nodules (*arrowheads*) in the white matter dorsal and lateral to the external angle of the lateral ventricles. **C,** Hematoxylin and eosin-stained section of a subcortical multifocal WMI shows multiple petechial hemorrhages that always surrounded a necrotic core lesion adjacent to a small vessel (*white arrows*). **D,** Hematoxylin and eosin-stained section of a petechial hemorrhage in which numerous leukocytes, which includes PMNLs, monocytes, and lymphocytes, were observed within or around the perivascular necrotic region. (Original magnifications: **A,** ×40; **C,** ×40; **D,** ×400.)

Saito. Inflammation and cerebral white-matter injury. Am J Obstet Gynecol 2009.

Second, in the histopathologic analysis of the fetal brain, 4 of the 5 fetuses (80%) in the exchange transfusion group of the current study exhibited focal WMIs (Table 3), although the brain total hemoglobin levels in this group never deteriorated from the baseline values throughout the study period (Figure 3, F) and none of the fetuses in the exchange transfusion group without intra-

uterine inflammation in our previous study exhibited PVL.[13,14] Interestingly, no significant differences in the frequency or severity (number of necrotic foci) of the focal WMIs were found between the 2 treatment groups in the present study (Table 3).

Third, in addition to PVL, subcortical multifocal WMIs were found in both the hemorrhage and exchange transfusion groups in the present study (Figure 4, B-D). Because the hemorrhagic lesions individually surrounded necrotic foci that were adjacent to a small vessel, serious ischemia in the fetal brain initially may have induced the necrotic foci, and subsequent postischemic reperfusion may have caused the hemorrhagic rupture of the injured small vessels. This cause of cerebral ischemia might have been more critical than that resulting from the PVL that was induced by hemorrhagic hypotension in our previous study because subcortical white matter generally maintains a more abundant perfusion than the periventricular region.

In this context, the sudden cessation of the fetal brain-sparing effect that was induced by the hemodynamic insults was suspected strongly to be the cause of the cerebral ischemia and the subsequent serious postischemic reperfusion injuries that frequently expanded from the periventricular medullary arterial territory to the subcortical area and resulted in PVL accompanied by subcortical multifocal WMIs. A few reports have studied the limitations of the fetal brain-sparing effect.[10,24] In a clinical study that analyzed Doppler velocimetry in the fetal middle cerebral artery, Fu and Olofsson[24] reported that fetuses with established brain-sparing blood flow had a limited capacity and a narrow margin for further increases in cerebral blood flow during superimposed acute hypoxic stress. The data in the present study were compatible with these previous observations and suggest the novel possibility that additional hypotension or hypoxia that exceeds the limit of the brain-sparing capacity might collapse the compensatory reaction itself; moreover, such abrupt changes in cerebral blood flow would cause critical brain ischemia, even if the fetus was capable of maintaining the cerebral blood volume over its initial baseline value.

On the other hand, the possibility of latent diffuse WMIs cannot be excluded in the present study. Indeed, inflammatory cytokines that are released from PMNLs in multifocal hemorrhagic lesions (Figure 4, D), increased, and activated in utero might stimulate microglia and macrophages directly around the lesions in a paracrine manner, which leads to the diffuse injury of premyelinating oligodendroglia in subcortical white matter. However, the infiltration of numerous reactive microglia in the periventricular white matter (reportedly a prominent feature of the early stages of diffuse WMI[25,26]) and the extensive infiltration of activated microglia and macrophages, the attenuation and fragmentation of axons, and the hypertrophy of reactive astrocytes in the subcortical white matter (reportedly seen in diffuse WMIs that have been induced experimentally in premature fetal sheep after exposure to intraamniotic endotoxin for 28 days[27]) were not observed in the present study, although immunohistochemical evaluations to distinguish changes in the density and distributions of each cell type were not performed. As a next research step, therefore, the initial changes that are associated with the diffuse WMIs that were induced in this experiment, compared with the white matter of fetuses without intrauterine inflammation, should be clarified by immunohistochemical methods.

Based on these considerations, we concluded that fetal G-CSF administration and intraamniotic endotoxin induced intensive intrauterine inflammation in a premature fetal sheep model. This intrauterine inflammation led to systemic changes in oxygenation and hemodynamics and triggered a compensatory increase in cerebral blood flow. Subsequent abrupt hypotensive or hypoxic insults appeared to terminate this compensatory increase that caused not only periventricular focal WMIs but also subcortical multifocal WMIs. Therefore, intrauterine inflammation so severe as to affect fetal cerebral hemodynamics may be an exacerbating factor in focal WMIs.

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シンポジウム3「脳性麻痺は防止できるか？」

子宮内炎症と神経細胞死

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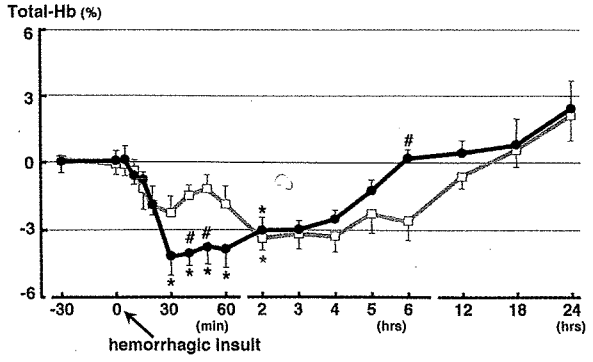
Key words
Funisitis
cerebral white matter injury
periventricular leukomalacia
near-infrared spectroscopy
brain-sparing effect

1. はじめに

早産低出生体重児に生じる脳白質損傷（white matter injury，以下WMI）は脳性麻痺をはじめとする神経学的後遺症の責任病変として重要である[1]．そこで，われわれは巣状WMIの本質的成因が低酸素ではなく循環不全にもとづく脳虚血であることを証明するために，先ず，ヒツジ胎仔に急性脱血性低血圧を負荷する脳室周囲白質軟化（periventricular leukomalacia，以下PVL）モデルを開発し[2) 3)]，次いで，近赤外線分光法（NIRS）を用いてその脳血流動態を解析することによって胎生期PVL発症時における脳虚血の病態生理学的特徴を明らかにした（図1）[4]．さらに，子宮内炎症がWMI発症に与える影響を解析するために，ヒツジ胎仔を用いた子宮内炎症モデルを作成し（図2），胎仔に顆粒球コロニー刺激因子（granulocyte-colony stimulating factor，以下G-CSF）を静注して血中多核白血球（polymorphonuclear leukocytes，以下PMNL）数を増加させ，さらに羊水腔にendotoxinを注入してこれらを局所で活性化させることによって，早産陣痛を誘発することなく壊死性の臍帯炎ならびに絨毛膜羊膜炎を誘導することができた[5]．

本研究の目的は，上記のヒツジ胎仔による慢性実験系を応用して，子宮内炎症がWMIに与える影響を解析することである．

図1　NIRSで解析した胎仔脳組織中total Hb濃度の経時的変化[4]．脱血群胎仔では急性脱血による全身性低血圧から回復した後（負荷後40分以降）も脳血液量の減少が遷延し（no re-flow phenomenon），負荷後6時間には交換輸血群に比較して脳血液量が有意に増加した（虚血後再灌流障害によるうっ血）．したがって，PVL発症時に生じる脳虚血の病態生理学的特徴は「血圧回復後の再灌流不全と引き続く脳組織のうっ血」と考えられた．縦軸は胎児脳組織中total Hb濃度の相対値（%で表示）．●：脱血群，□：交換輸血群．*p＜0.05（Dunnett test）：各群内における負荷前値との比較．#p＜0.05（Wilcoxon signed-rank test）：負荷後6時間における2群間での比較．



2. 対象と方法

本研究は東北大学動物実験委員会の承認のもと（No. 15-128），東北大学医学部附属動物実験施設にて平成16年11月から平成18年3月にかけて実施された（文科省科研費課題番号16591702，18591213）．

対象は妊娠期間を確定したSuffolk種ヒツジ胎仔15頭．慢性実験系を作成するために，妊娠102-103日（満期147日）に全身麻酔下に母獣を開腹して子宮切開し，胎仔の腹部大動脈と上下大静脈，羊水腔内にカテーテルを留置，頭頂骨にNIRS用ライトガイドを固定した後，胎仔を子宮内にもどして閉腹した[4]．以後，胎仔の心拍数，血圧ならびに羊水内圧波形を連続監視した．胎仔胎盤系の循環血液量を算出するために，母仔ともに全身状態が安定した妊娠104-105日に胎仔に凍結新鮮血漿を用いた交換輸血を実施した[2) ~4)]．子宮内炎症を誘導するために，妊娠105日からすべての胎仔

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図2 G-CSFの静脈内投与とendotoxinの羊水内投与によって胎仔に誘導された壊死性の臍帯炎（a and b）ならびに絨毛膜羊膜炎（c）の組織病理学的所見[5].
大量のPMNLが血管内腔（v）から羊水腔（af）に向かって遊走浸潤しており，壊死した多量のPMNLが好塩基性沈着物とともに（矢印），臍帯では臍帯動脈壁の外周に円環状に蓄積し（a and b），卵膜では羊膜直下の絨毛膜内に集積していた（c）．いずれもhematoxylin-eosin染色で，拡大率はそれぞれa：x40，b：x400，c：x100.

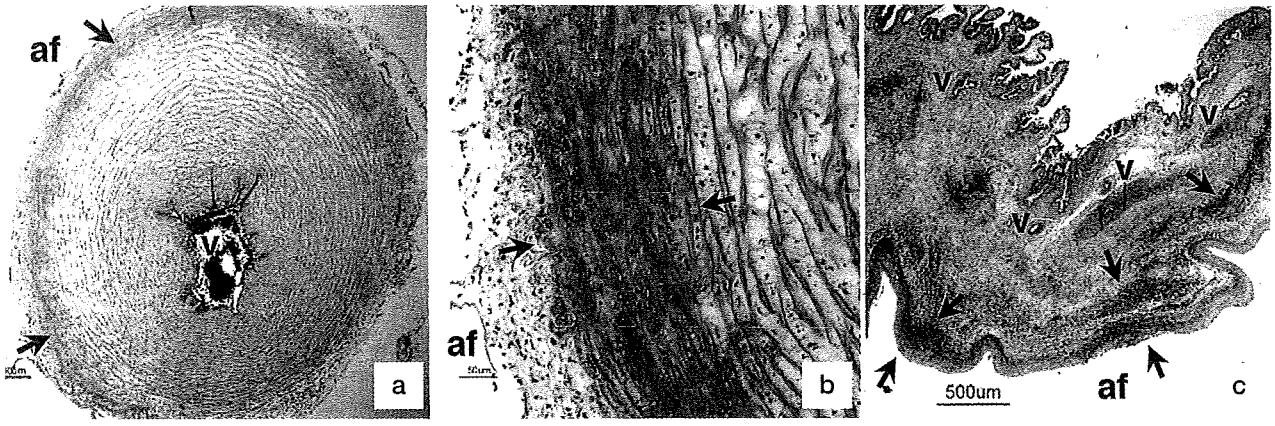
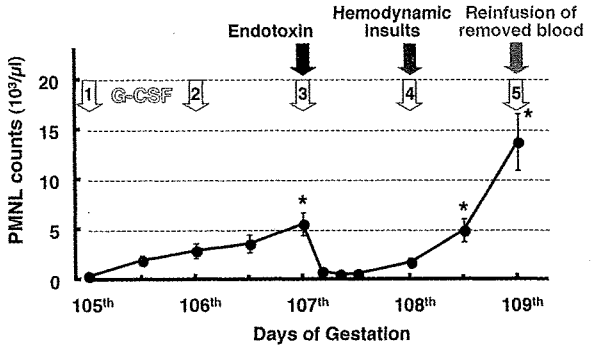


図3 胎仔腹部大動脈血中PMNL数の経時的変化.
PMNL数は羊水腔内endotoxin注入直後の24時間には一過性の減少を示したものの，妊娠107日以降にはG-CSF投与前値に比較して有意な増加が観察された．データはすべて「平均±標準誤差」で表示．*p＜0.05（Dunnett test）：妊娠105日のG-CSF投与前値との比較.



にG-CSF（ノイトロジン®，中外製薬）40 μg/日を5日間静注し，妊娠107日には羊水腔内にendotoxin（E.coli 055：B5，Sigma Chemical Co）20 mgを注入した（図3）[5].

羊水腔内にendotoxinを注入した24時間後（妊娠108日）に胎仔を3群（各n＝5）に分け，それぞれに循環負荷実験を実施した[2)~4)]．脱血群には循環血液量の約40％を急性脱血して全身性低血圧を負荷，交換輸血群では循環血液量の約40％を凍結新鮮血漿と交換輸血して脱血にともなう貧血性低酸素のみを負荷し，それぞれ負荷後24時間から5時間かけて戻し輸血した．対照群には循環負荷は実施しなかった．羊水腔endotoxin注入前3時間から循環負荷実験終了まで，胎仔脳組織中Hb濃度の変動をNIRSにて連続解析した[4]．羊水腔endotoxin注入6日後（妊娠113日）に帝王切開し，胎仔脳を10％中性ホルマリン緩衝液で灌流固定して組織病理学的解析に供した.

統計学的検討にはDunnett test，Kruscal-Wallis test，Scheffe test，repeated-measures ANOVA，Wilcoxon signed-rank testを用い，連続データは「平均値±標準誤差」で表してp＜0.05を有意差ありとした.

3. 成績

実験期間中の胎仔血中PMNL数の経時的変化を図3に示した．PMNL数は羊水腔内endotoxin注入後24時間には一過性の減少を示したものの，妊娠107日以降にはG-CSF投与前値に比較して有意な増加が観察された.

子宮内局所に炎症が誘導され始めたendotoxin注入後の24時間には，胎仔血中oxygen contentの持続的減少とlactate濃度の一過性増加（図4，A），および脳組織中deoxy-Hbならびにtotal Hb濃度の持続的増加（図4，B）が認められた.

三群間（脱血群，交換輸血群，対照群）における胎仔基礎データ（循環血液量，脱血量，体重，脳重）の比較ではいずれも有意な差は認められず，循環負荷実験直前の生理学的パラメータ値（心拍数，平均動脈圧，血液ガス分析値，血中のPMNL数，Hbおよびlactate濃度，oxygen content，脳組織中Hb濃度）の比較でも有意な差はなかった（Kruscal-Wallis test）.

脱血群ならびに交換輸血群における循環負荷中の平均動脈圧と脳組織中total Hb濃度の経時的変化にはいずれも対照群のそれと比較して有意な差が認められた（p＜0.05，repeated-measures ANOVA）．平均動脈圧は脱血群では負荷開始後20分まで急激に減少してその後は速やかに自然回復したが（図5，A），この間に交換輸血群では一過性で軽度の頻脈が観察された．負荷実験前の脳組織中total Hb濃度は脱血群で11.3±2.8％，交換輸血群で9.7±3.9％といずれも持続的に増加して

図4　羊水腔内endotoxin注入後の24時間における胎仔血中oxygen contentならびにlactate濃度（a），および脳組織中Hb濃度（b）の経時的変化．

胎仔血中oxygen content（a：●）は羊水腔内endotoxin注入後12時間から持続的に減少し，lactate濃度（a：□）はendotoxin注入後12時間に一過性に増加した．脳組織中oxy-Hb濃度（b：■）に有意な経時的変化は認められなかったが，deoxy-Hb（b：□）ならびにtotal Hb濃度（b：●）はendotoxin注入後9時間から持続的に増加した．データはすべて「平均±標準誤差」で表示．*p＜0.05（Dunnett's test）：妊娠107日のendotoxin投与前値との比較．

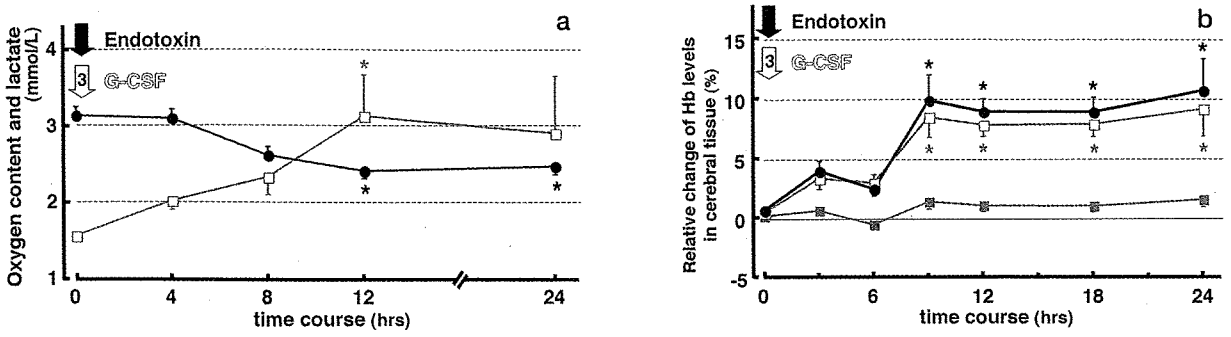
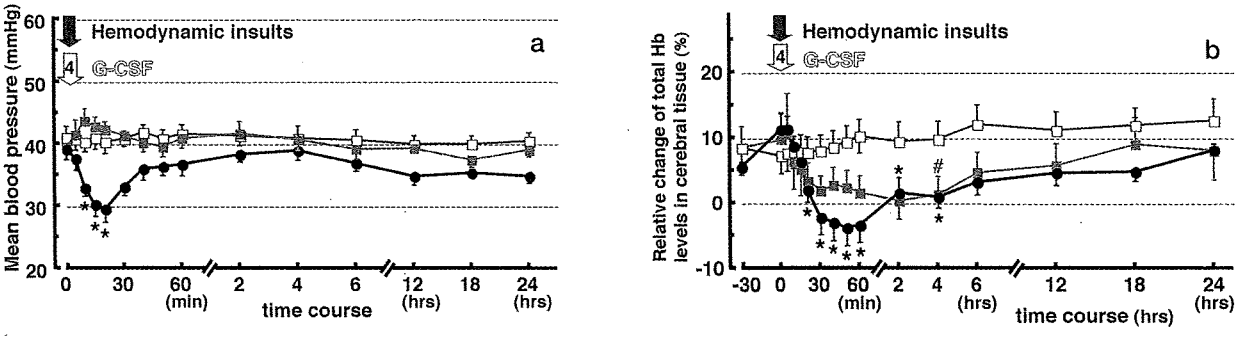


図5　脱血群，交換輸血群，対照群における循環負荷中の平均動脈圧（a）と脳組織中total Hb濃度（b）の経時的変化の比較

脱血群の平均動脈圧（a：●）は負荷開始後10～20分まで急激に減少してその後速やかに自然回復したが，交換輸血群（a：■）ならびに対照群（a：□）では有意な変動は観察されなかった．脱血群の脳組織中total Hb濃度（b：●）は負荷後20分~4時間まで最大幅-15.2±2.8％の減少が観察され，交換輸血群（b：■）では対照群（b：□）と比較して有意な減少は負荷後4時間のみであったが最大幅-10.4±4.9％の減少が観察された．データはすべて「平均±標準誤差」で表示．*p＜0.05（Dunnett test）：妊娠108日の循環負荷開始前値との比較．#p＜0.05（Scheffe test）：負荷後4時間における3群間での多重比較．



いたが，脱血群では負荷開始後20分から4時間まで最大幅-15.2±2.8％の減少が観察され，交換輸血群では対照群と比較して有意な減少は負荷後4時間のみであったが最大幅-10.4±4.9％の減少が観察された（図5，B）．

胎仔付属器における組織病理学的検索の結果，15例すべての胎仔において図2と同様の壊死性臍帯炎ならびに絨毛膜羊膜炎が観察された．胎盤分葉では羊膜上皮に炎症細胞浸潤が認められたが，絨毛血管壁には明らかな炎症性変化は認められなかった．

胎仔中枢神経系における組織病理学的検索の結果を三群間で比較して表1に示した．脱血群の4例と貧血群の4例にそれぞれPVLが観察されたが（図6，A），一頭当たりの白質軟化巣数（径＞500 μm）では両群間に明らかな差はなく，対照群では中枢神経系病変は認められなかった．また，脱血群の1例と貧血群の2例にはPVLのみならず皮質下白質を主座とする多発性小出血壊死巣が観察された（図6，B）．多くの小出血巣ではその中心に小血管に隣接する壊死病変が認められ（図6，C），壊死巣内には多核好中球と単核球が多数含まれていた．

表1　脱血群，交換輸血群，対照群における脳白質損傷所見の比較

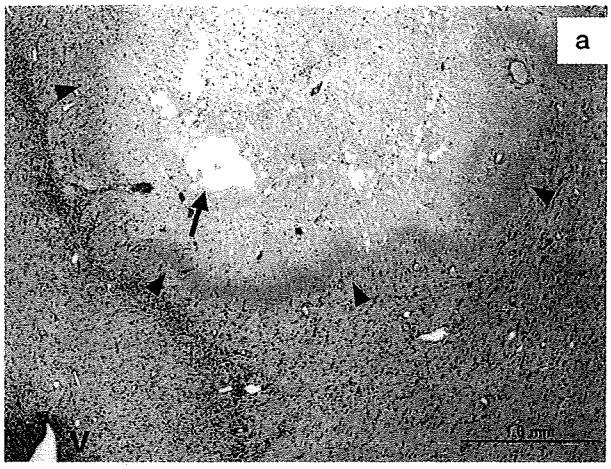
	脱血群 (n＝5)	交換輸血群 (n＝5)	対照群 (n＝5)
PVL*	4	4	0
一頭当たりの白質軟化巣数*	3.0±1.8	3.0±1.4	0.0±0.0
皮質下多発性小出血壊死	1	2	0

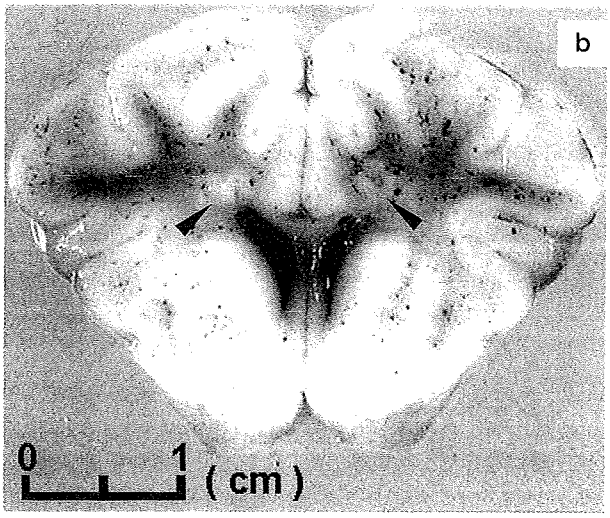
離散量は群内の陽性頭数で，連続量は「平均±標準誤差」で表した．「白質軟化巣数」では径500 μm以上の軟化巣を計数した．
*p＜0.05（Kruscal-Wallis test）．

4. 考察

本実験では胎仔にG-CSFを静注して血中PMNL数を

図6　脱血群ならびに交換輸血群で認められた脳白質損傷の組織病理像.
a：交換輸血群に認められたPVLの組織像．線条体レベルの冠状断において側脳室（v）外背側の深部白質に凝固壊死巣が観察され（矢頭），病変内部は空洞形成されつつあった（矢印）．**b**：交換輸血群に認められた皮質下白質を主座とする多発性小出血壊死巣の前基底核レベル冠状断面における肉眼像．PVLは小白色結節として認められた（矢頭）．**c**：多発性小出血壊死巣の組織像．大多数の小出血病変ではその中心に小血管に隣接する壊死巣が観察された（矢印）．いずれもhematoxylin-eosin染色で，拡大率はそれぞれA：x40，B：x1，C：x40.





増加させ，さらに羊水腔内にendotoxinを注入してこれらを局所で活性化させることにより，強度の子宮内炎症である壊死性の臍帯炎ならびに絨毛膜羊膜炎を誘導することができた．Endotoxin投与直後の24時間に観察された一過性の血中PMNL数減少は（図3），臍帯ならびに卵膜において大量のPMNLが血管内腔から羊水腔に向かって遊走浸潤した結果を反映したものと考えられた[5]．

同時にこの期間には胎仔血中oxygen contentの持続的減少とlactate濃度の一過性増加（図4，A）が認められ，強度の子宮内炎症は胎仔に低酸素症を誘導する可能性が示された．すなわち臍帯や卵膜に生じた広範で強い血管炎が胎盤血管まで波及して血管攣縮や間質の浮腫が生じ，その結果として胎盤血管でのガス交換が機能的可逆的に障害された可能性が示唆された[6) 7)]．一方，このとき全身性には低酸素症が誘導されていたにもかかわらず，NIRSによる脳血流解析上では代償性の脳血流量増加によって胎仔脳への酸素供給が維持されていた．血中oxygen contentは明らかに減少したにもかかわらず脳組織中oxy-Hb濃度が低下しないばかりか，deoxy-Hbならびにtotal Hb濃度が持続的に有意な増加を示しており（図4，B），これはbrain-sparing effect（以下，BSE）と呼ばれる臓器血流の再分配にもとづく代償性の脳血流増加によるものと推察された[8) 9)]．

胎仔脳にBSEが誘導されている状況において引き続き循環負荷実験を実施したところ，以前に報告した非炎症下での循環負荷実験（図1）[4)]とは異なる興味深い結果が三点得られた．

第一の相違点は循環負荷によって生じた脳組織中total Hb濃度における減少の程度にあった．本実験の脱血群（-15.2±2.8％）ならびに交換輸血群（-10.4±4.9％）における循環負荷直後の最大減少幅（図5，B）はいずれも非炎症下実験における脱血群（-4.2±0.8％）ならびに交換輸血群（-3.3±0.5％）の最大減少幅（図1）に比較して有意に大きかった（$p<0.05$；Wilcoxon signed-rank test）．両実験で認められた減少幅の差は本実験において循環負荷前に観察されていたBSEによる脳組織中total Hb濃度の増加分に匹敵しており，本実験では両群ともに循環負荷を契機として急激にBSEが機能しなくなった可能性が示唆された．

第二の相違点は，交換輸血群では非炎症下においてこれまで決してPVLが誘導されず[2) 4)]，本実験においても脳組織中total Hb濃度は決して基線以下には減少しなかったにもかかわらず（図5，B），その80％の胎仔脳にPVLが誘導されたことである．脱血群と交換輸

血群の間でPVLの発症頻度とその程度に差が認められなかったことから（表1），両群胎仔に生じた脳虚血はおそらく同じ原因によって誘導されたものと推察された．

第三の相違点は本実験では両群ともその20～40％の胎仔脳においてPVLに加えて皮質下白質に多発性小出血壊死巣が認められたことである（図6，BとC）．大多数の出血病変が小血管に接した壊死巣を取り囲んでいたことから，胎仔脳に生じた脳虚血が髄質動脈周囲に虚血病変を誘導した後，引き続く虚血後再灌流によって小血管壁が破綻して出血したのかも知れない．さらに，皮質下白質は一般的に脳室周囲白質に比較して血流量が豊富であるため，本実験で誘導された脳虚血は非炎症下実験でPVLを誘導した脳虚血に比較してより重篤であった可能性が高い．

したがって，本実験では循環負荷そのものによる低血圧や低酸素ではなく，循環負荷を契機として突然BSEが解除されたことこそが重篤な脳虚血ならびに虚血後再灌流が誘導された原因であり，その結果，脱血群のみならず貧血のみ負荷された交換輸血群にも初めてPVLおよび皮質下白質の多発性小出血壊死巣が誘導されたものと推察された．FuとOlofssonは子宮内発育遅延と診断された胎児の中大脳動脈血流速の波形を解析して[10]，いったんBSEが確立された後では，さらなる低酸素ストレスに対して胎児が脳血流を増加させる能力には限界があり，少ない余力しか有していないことを指摘した．本実験の結果はこうした臨床報告と矛盾しないばかりか，BSE予備能を越えるような循環動態の変動が急激に生じた場合には胎児はこれに適応できず，その代償反応自体が消滅して重篤な脳虚血が誘導されるという新たな可能性を指摘できた．

以上の考察にもとづいて，胎仔へのG-CSF静注と羊水内endotoxin注入によって誘導された強度の子宮内炎症のもとでは，胎盤炎症による低酸素症に対して代償性に作動した胎仔脳血流量の増加反応は低血圧や低酸素による循環変動によって破綻しやすく，その結果生じた脳虚血によって未熟な胎仔脳に脳室周囲白質の巣状WMIのみならず皮質下白質に多巣性WMIが引き起こされる可能性が高いとわれわれは結論した．したがって，胎仔の脳循環動態に影響するほどの子宮内炎症は巣状WMI発症に対する増悪因子の一つと考えられた．

5. おしまいに

早産低出生体重児に生じるWMIの病態を解明する道程は遠く，これを予防するための有効な方法を開発するには現状はまだほど遠いレベルにあると言わざるを得ない．現在，われわれは上記の研究成果を踏まえて，その追加実験として「子宮内炎症ではなく母獣への低酸素負荷によって胎仔に誘導されたBSEにおいてWMIを検討」「母獣への酸素投与によるWMI予防効果を検討」「母獣への副腎皮質ステロイド投与によるWMI予防効果を検討」「microglia/macrophage，astrocyte，oligodendrocyte系幼若細胞の分布動態を画像解析することによって皮質下白質のびまん性WMIを検索」している．これからも本学会会員によって胎児WMIに関する多くの臨床ならびに基礎研究が発展することを期待している．

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