Fetal cells in the maternal appendix: a marker of inflammation or fetal tissue repair?

Margarida Avo Santos1,6, Keelin O'Donoghue1,2,3, Josephine Wyatt-Ashmead4 and Nicholas M. Fisk1,2,5

1Division of Surgery, Oncology, Reproduction and Anaesthesia, Faculty of Medicine, Institute of Reproductive and Developmental Biology, Imperial College London, UK; 2Centre for Fetal Care, Queen Charlotte's and Chelsea Hospital, London, UK; 3Department of Obstetrics and Gynaecology, University College Cork and Cork University Maternity Hospital, Cork, Ireland; 4Department of Pathology, Hammersmith Hospitals Trust, Du Cane Road W12 0NN, London, UK; 5University of Queensland Centre for Clinical Research, Brisbane, Australia

6Correspondence address: Department of Reproductive Medicine and Gynaecology, University Medical Centre Utrecht, PO Box 85500, 3508 GA, Utrecht, The Netherlands. E-mail: m.daavoribeirodossantos@umcutrecht.nl

BACKGROUND: Fetal microchimeric cells that have trafficked into the maternal circulation persist in maternal tissues for years after pregnancy, but their biological role is unclear. We investigated whether fetal cells participate in maternal tissue repair during human pregnancy. METHODS: Appendix specimens were acquired from women undergoing appendicectomy during \((n = 8)\) or after \((n = 1)\) pregnancy. Fluorescence in situ hybridization (FISH) determined the presence of male presumed-fetal cells, and immunostaining indicated the fetal cell phenotype. RESULTS: Male cells were identified in appendiceal tissues from all women with known present or past male pregnancies \((n = 7)\) and from a woman with a previous spontaneous abortion of undetermined gender \((n = 1)\), but not in one woman with three daughters. One woman was only 6 weeks pregnant at appendicectomy. Male cells were evenly distributed through appendix tissues, in larger numbers where there was a greater degree of inflammation and when the current pregnancy was male. Combined immunostaining and Y-FISH demonstrated male desmin+ muscle cells and CD3+ lymphocytes, suggesting fetal cells had differentiated. CONCLUSIONS: Male-presumed fetal cells of haematopoietic and mesenchymal origin were identified in the appendix of all pregnant women who had sons. We suggest that fetal cells are present at sites of maternal tissue injury during pregnancy, and may participate in tissue repair.

Keywords: fetal cell microchimerism; pregnancy; appendix; fetal cells; tissue repair

Introduction

Fetal cell microchimerism (FCMC) is characterized by the presence of rare event fetal cells in the mother and results from traffic of fetal cells across the placenta as occurs in every pregnancy (Jackson, 2003; O’Donoghue et al., 2004). Fetal cells enter the maternal circulation from early in gestation (four to six post-menstrual weeks) and engraft maternal tissues where they persist for decades (Thomas et al., 1994; Bianchi et al., 1996; Ariga et al., 2001; O’Donoghue et al., 2004).

FCMC was originally implicated in the pathogenesis of autoimmune diseases such as systemic sclerosis (Nelson, 2002). However, this hypothesis has been weakened by discovery of persistent fetal cells in non-autoimmune diseases such as hepatitis C, thyroid adenomas and cervical cancer (Khosrotehrani and Bianchi, 2003). Since fetomaternal trafficking occurs in all pregnancies (Ariga et al., 2001; Krabchi et al., 2001), FCMC is now believed to be established in healthy women as well (Bianchi et al., 1996; Adams et al., 2003). Older largely PCR-based studies showed a wide range in the frequency of FCMC in healthy women (median 11%, range 0–72%, O’Donoghue, 2008, unpublished observations). More recently, animal studies have suggested very high rates of FCMC (Khosrotehrani et al., 2007,2008), while limited human studies suggest most if not all pregnant women harbour persistent fetal cells in their tissues for decades after pregnancy (Khosrotehrani et al., 2004; O’Donoghue et al., 2004).

The fetal cell type responsible for FCMC is unknown, but candidates include all cells in fetal blood that persist long-term (O’Donoghue, 2006). Evidence from human and animal studies shows clustering of microchimeric fetal cells around sites of maternal tissue injury, implicating stem cells (Srivatsa et al., 2001; Khosrotehrani et al., 2004; O’Donoghue et al., 2004; Nguyen Huu et al., 2007); these could be mesenchymal,
The biological role of persistent fetal cells is unclear. Limited evidence suggests that they participate in tissue repair. Animal studies implicate FCMC in tissue repair rather than disease pathogenesis. In rodent models, increased microchimeric fetal cells have been found clustered at sites of skin fibrosis (Christner et al., 2000) chemical hepatic/renal injury (Wang et al., 2004; Khosrotehrani et al., 2007), and neural injury (Tan et al., 2005). Green fluorescent protein-labelled fetal cells were found in maternal sites of skin inflammation during murine pregnancy using a transgenic reporter for endothelial markers, suggesting fetal cells participated in maternal angiogenesis during pregnancy (Nguyen Huu et al., 2007). In humans, male cells have been identified in post-reproductive women with cervical cancer and thyroid tumours (Srivatsa et al., 2001; Cha et al., 2003; Dubernard et al., 2008). However, the only direct evidence in humans for FCMC participating in tissue repair after pregnancy is the detection of microchimeric male cells bearing epithelial, leukocyte or hepatocyte markers in diseased tissue, which were absent from male cells in adjacent healthy tissue (Khosrotehrani et al., 2004). We recently reported FCMC in healthy and diseased lung tissue from post-reproductive women with a 7-fold greater frequency of male microchimeric cells in lung than in bone marrow. Male cells were clustered in diseased rather than surrounding healthy tissues, implicating microchimeric cells in the repair process (O’Donoghue et al., 2008). To date, there has been no direct evidence in humans for microchimeric fetal cells participating in tissue repair during pregnancy. Indirectly, Aractingi et al. (1998) detected male DNA in 10 skin biopsies from pregnant women with the non-specific skin lesion of polymorphic eruption of pregnancy, but did not determine the cell phenotype. More recently, fetal cells were identified in breast cancer stromal tissue from pregnant women, suggesting their possible influence over tumour behaviour (Dubernard et al., 2008).

Human studies of FCMC during pregnancy should offer insight into both its role in tissue repair in pregnancy, and the timing of establishment of microchimerism, as well as providing a useful tool to investigate its role in the altered inflammatory response during pregnancy. Therefore, in this study, we aimed to investigate whether fetal cells were involved in tissue repair in women during pregnancy, using tissue from a cohort of women pregnant with male fetuses who underwent appendectomy as a model of injured tissue in pregnancy.

Materials and Methods

Subjects

Surgical appendix specimens removed during pregnancy were collected from the Hammersmith Hospital tissue bank, after project approval from the Research Ethics Committee of Hammersmith and Queen Charlotte’s National Health Service (NHS) Trust (REC 2002/6335). We used specimens obtained where the patient had ticked the consent box on the NHS form allowing research on stored tissues. Delivery and neonatal outcome data (Table I) were obtained from the hospital Integrated Computerized Hospital Information System.

Tissue preparation

Tissues had initially been preserved in 10% neutral buffered formaldehyde (Sigma–Aldrich Co. Ltd), and specimens processed to paraffin wax cut into serial 3 μm sections mounted on polylysine-coated slides. Blocks of appendix tissue were obtained, and sections from each block chosen at random for analysis. Each slide contained two cut sections of the appendix. Therefore, two areas (approximate size 13 x 11 mm) per slide, corresponding to two different cut sections, were selected for analysis. Representative slides from each tissue block were stained with conventional haematoxylin and eosin (H&E) for analysis by light microscopy (CX-40, BX-50, Olympus UK Ltd.), to delineate appendiceal pathology and co-localize microchimeric (male) cells identified by fluorescent in situ hybridization (FISH) in healthy and diseased tissues. Slides were reviewed by a histopathologist (J.W.A), who confirmed the relevant pathologic aspects of each appendix specimen.

FISH

FISH on paraffin-embedded tissues was modified from published methods (Johnson et al., 2000; Steel et al., 2005), as described previously (O’Donoghue et al., 2008). The chromosome-specific centromeric repeat probes DXZ1, labelled with SpectrumOrange™ and DYZ1, with SpectrumGreen™ (Vysis, Abbott Laboratories Ltd., Maidenhead, UK) were used as standard FISH probes. Female and male paraffin-embedded neonatal appendix sections were negative and positive hybridization controls, respectively. Placental and umbilical cord tissue samples were also used as positive controls to make sure there was no non-specific signalling of the probes used in this study. FISH was repeated using an additional chromosome-specific centromeric repeat probe DYZ1 labelled with SpectrumOrange™ (Vysis), which was applied to randomly selected slides from each block.

Cell number quantification

Dewaxed tissue sections were scraped with sterile scalpels and placed into 1.5 ml microcentrifuge tubes for genomic DNA isolation using the QIAamp DNA Micro Kit (Qiagen Ltd., Crawley, UK). DNA extraction was performed according to the manufacturer’s recommendations and eluted from the column using 40 μl diethylpyrocarbonate-treated water. The amount of double-stranded DNA (μg/μl) extracted from each section was quantified by spectrophotometry (BioPhotometer, Eppendorf AG, Hamburg, Germany). Assuming that each nucleated cell contains, on average, 5 pg of DNA (Bianchi et al., 2001), the number of cells per section was determined according to the amount of DNA isolated.

Immunohistochemistry

For immunohistochemistry, dewaxed tissue sections were pre-treated first with 2 × standard saline citrate for 15 min at 80°C and then 100 μl of 100 μg/ml pre-warmed protease K (pK) (20 mg/ml; Roche) for 10 min at 37°C and finally fixed with ice-cold 2:1 v/v methanol: acetone for 2 min. When air-dried, slides were incubated for 1 h with 100 μl of block [5% normal goat serum (NGS, Sigma–Aldrich Company Ltd.) and 5% bovine serum albumin (BSA, Sigma–Aldrich) in phosphate-buffered saline (PBS)]. The slides were washed twice in PBS, 3 min each, and then incubated for 1 h with 100 μl of the appropriate anti-human murine immunoglobulin (IgG)-class monoclonal antibody diluted in 5% BSA in PBS (desmin 1:50 or CD3 1:25, DakoCytomation, Cambs., UK). Sections were washed twice in PBS, 3 min each, and then incubated with

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### Table I. Male microchimeric cells in appendiceal specimens from women.

<table>
<thead>
<tr>
<th>Code</th>
<th>Age (years)</th>
<th>Histology</th>
<th>Sections analysed</th>
<th>Male cells detected (%)</th>
<th>Total no. cells</th>
<th>Ratio (male cells/total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>29</td>
<td>Acute appendicitis</td>
<td>14</td>
<td>0</td>
<td>14,000</td>
<td>0.92 x 10^6</td>
</tr>
<tr>
<td>A2</td>
<td>26</td>
<td>Fibrosis and congestion</td>
<td>16</td>
<td>4</td>
<td>1,680,000</td>
<td>1.88 x 10^6</td>
</tr>
<tr>
<td>A3</td>
<td>21</td>
<td>—</td>
<td>1</td>
<td>22</td>
<td>839,000</td>
<td>10.0 x 10^6</td>
</tr>
<tr>
<td>A4</td>
<td>24</td>
<td>Fibrosis and congestion</td>
<td>9</td>
<td>15</td>
<td>1,220,000</td>
<td>1.0 x 10^6</td>
</tr>
<tr>
<td>A5</td>
<td>28</td>
<td>TOP</td>
<td>1</td>
<td>17</td>
<td>2,392,000</td>
<td>9.0 x 10^6</td>
</tr>
<tr>
<td>A6</td>
<td>27</td>
<td>—</td>
<td>1</td>
<td>6</td>
<td>2,936,000</td>
<td>1.0 x 10^6</td>
</tr>
<tr>
<td>A7</td>
<td>33</td>
<td>—</td>
<td>1</td>
<td>21</td>
<td>3,936,000</td>
<td>1.0 x 10^6</td>
</tr>
<tr>
<td>A8</td>
<td>23</td>
<td>Chronic appendicitis</td>
<td>11</td>
<td>13</td>
<td>5,344,000</td>
<td>1.0 x 10^6</td>
</tr>
<tr>
<td>A9</td>
<td>28</td>
<td>Acute appendicitis</td>
<td>7</td>
<td>22</td>
<td>3,544,000</td>
<td>1.0 x 10^6</td>
</tr>
</tbody>
</table>

**Imaging and analysis**

Following FISH and/or immunohistochemistry, slides were analysed by epifluorescence microscopy (Zeiss Axioskope, Germany), using single band pass filters for Aqua, Orange and DAPI and the triple band pass filter set. Images were captured using a cooled CCD camera, reviewed in Quipps m-FISH software (Vysis). Nuclei with two red signals were categorized as female (XX) and those with one red and one green as male (XY). Each slide was checked for hybridization efficiency and analysed if >75% of the nuclei showed two signals. Only fluorescence signals of approximately equal size inside an intact nucleus indicated by DAPI were accepted (Johnson et al., 2000; O'Donoghue et al., 2004). To distinguish true signals from tissue autofluorescence, image intensities were captured and compared under all filter channels using high magnification (100×). Each slide was examined twice: an initial scan through the tissue and a later more detailed survey of all signals. Areas within sections were ignored if poor hybridization or over-digestion was observed. Counting of Y-positive nuclei was accomplished by systematically examining the tissue, field by field, under 100× magnification. To reduce any bias, the primary investigator (MAS) was blind to the reproductive histories throughout analysis and a second observer (KOD), who was not blinded, later confirmed the scoring of male cells (O'Donoghue et al., 2008). Non-parametric statistics were used and categorical data compared by Fisher exact test, with $P < 0.05$ considered significant.

### Results

Appendix sections were obtained from eight women who underwent appendicectomy during their pregnancy and from one woman where the appendix was removed 17 days post-partum. These women were entirely healthy prior to surgery and had never had a transfusion or organ transplant. Specimens were coded (A1–9) to maintain anonymity. The median age of the nine women was 27 years (range 21–38), and only one woman had never delivered a son nor had an early pregnancy loss; her specimen was the sole negative control. Five women were pregnant with male fetuses at the time of appendicectomy and two of the remaining three with female fetuses had previously delivered sons (Table I). The other woman with a female fetus had never had a full-term male pregnancy but
had a history of early pregnancy loss. Based on the different reproductive histories, we categorized the nine patients into four different groups: no male pregnancies (A1); possible previous male pregnancy (A4); current male pregnancy (A3, A5, A6, A7 and A8); and previous male pregnancy (A2 and A9).

Three hundred and nine slides from 21 blocks of appendix tissue were obtained from the Hammersmith Hospital tissue bank. Although appendicectomies were performed for a clinical diagnosis of acute appendicitis, not all removed appendices showed evidence of inflammation. This is consistent with experience outside pregnancy, where just over a quarter of patients with suspected appendicitis have histologically normal appendices (Nemeth et al., 2001). Pathological review of all four layers of each appendix specimen (outer longitudinal smooth muscle, inner circular smooth muscle, submucosa and mucosa (Fig. 1A)) in H&E slides against the established diagnostic criterion of acute appendicitis (neutrophilic infiltration of the muscularis (Fig. 1B)) resulted in four different histological diagnoses. Three (34%) women had acute appendicitis, one (11%) early superficial appendicitis, two (22%) fibrosis and congestion, one (11%) resolving appendicitis and another (11%) had chronic inflammation of the appendix. Thus, 3/9 (34%) appendices overall showed no inflammation (A2, A3, A4).

A total of 105 male cells were detected in the 125 sections analysed, with 240 000–1 072 000 nuclei examined per section and a median of 14 (range 9–19) sections analysed per case. Male cells were identified in appendiceal tissue from all women with known current or previous male pregnancies (A2, A3, A5, A6, A7, A8, A9; n = 7), but not in the control (A1) without sons. Male cells were also found in one woman (A4) with one spontaneous abortion (gender unknown) and no sons. Therefore, male cells were located in 8/8 cases where expected (A2, A3, A4, A5, A6, A7, A8, A9) but not in the control with no sons (A1) (8/8 versus 0/1) (Table I).

The frequency of microchimerism observed in the appendix ranged from 1/133 454 to 1/1 084 000 (median 1/533 333). Table I suggests an association between reproductive history and the frequency of fetomaternal microchimerism. Women with current male pregnancies (A3, A5, A6, A7, A8) had the highest rates of appendicel microchimerism, followed by a woman with no sons but with a previous spontaneous abortion (A4) and finally by women with current female pregnancies that had sons previously (A2, A9). However, non-parametric unpaired statistics (P = 0.076) showed that this difference was not significant. Empirically, there appeared to be an association between the degree of inflammation at the time of appendicectomy and the frequency of FCMC. However, the correlation between microchimeric fetal cell number and degree of inflammation was not significant (Spearmann 0.6 P = 0.12). As summarized in Table I, the appendicel samples shown to be inflamed (A5, A6, A7, A8, A9) had higher numbers of microchimeric cells than the ones showing no inflammation (A2, A3 and A4), but the difference was also not statistically significant (P = 0.11). Small study numbers may have limited statistical analysis.

Co-localization of male cells identified by XY-FISH with H&E staining showed XY cells were evenly distributed in the different layers of the sections, being present in either muscle (56%) or mucosa and submucosa layers (44%). Morphology and co-localization of male cells by XY-FISH with H&E staining allowed identification of three types of male cells: muscle cells, lymphocytes and gland cells. Only a small portion (4%) corresponded to gland cells. The majority of male presumed fetal cells identified were phenotypically either muscle cells (55%) or lymphocytes (41%). The difference between the number of muscle and lymphocyte male cells observed was not significant.

XY-FISH using the DXZ1/DYZ1 probe was used as standard, but to confirm the presence of male cells in cases with known male pregnancies, an alternative probe for the Y chromosome was used. This single DYZ1 probe labelled with SpectrumOrange™ allows identification of only male cells. The median number of sections analysed per case for DYZ1 was 1 (range 1–2), and the number of sections analysed less (n = 19) than the number analysed by XY-FISH (n = 125). This is because Y-FISH was mainly used to confirm the presence of male cells in specimens in which male cells had previously been visualized, but also to allow later development of the combined FISH and immunohistochemistry method.

We tested anti-CD3, -CD19, -CD34 and -CD45 antibodies as haematopoietic markers and anti-desmin and -vimentin antibodies as mesenchymal markers. However, due to poor quality of the immunostaining on archival blocks using some of these antibodies, only anti-CD3 and -desmin gave sufficiently specific uptake to allow combining immunohistochemistry and FISH. We were able to identify cells with a Y chromosome with either haematopoietic (CD3+) (n = 14, range 1–7) or mesenchymal (desmin+) (n = 14, range 1–6) markers (Fig. 1G–H).

Discussion

In this study, we report the identification of male cells of presumed fetal origin in surgical specimens removed during pregnancy. We identified male cells in a cohort of women with known male pregnancies but also in a woman with no sons and a previous spontaneous abortion of undetermined gender. Analysis of cell morphology using co-localization techniques suggested that male cells identified had differentiated into muscle (desmin+) or T-lymphocytes (CD3+). This suggests that fetal microchimeric cells transferred into maternal blood during pregnancy can engraft maternal organs such as the appendix and implicate fetal microchimeric cells in tissue repair.

Previous reports attempting to demonstrate possible involvement of microchimerism in tissue repair in humans used specimens from post-reproductive women to show persistence of fetal cells in injured maternal tissues many years after delivery (Srivatsa et al., 2001; Khosrotehrani et al., 2004; O’Donoghue et al., 2008). In this study, we showed for the first time the presence of male cells of presumed fetal origin in the appendix of pregnant women diagnosed with appendicitis and investigated whether fetal cells participate in tissue repair during pregnancy. Our results confirm that during pregnancy microchimeric cells
cross the placental barrier to enter the maternal circulation as early as 6 weeks of gestation, as one woman (A6) was only 6 weeks pregnant at the time of her appendicectomy.

Comparing these data with our previous work on bone marrow and lung tumours from post-reproductive women, we observed less FCMC in the appendix compared with other tissues (O’Donoghue *et al.*, 2004, 2008). The frequency of microchimeric cells was 2-fold lower in the appendix tissues than in bone marrow (1/133 454 in appendix versus 1/70 000 cells in marrow) and 8-fold smaller than in diseased lung (1/133 354 in appendix versus 1/16 861 cells in lung). It has been previously suggested that the length of time elapsed since completion of pregnancy might affect the degree of microchimerism in women, as some studies reported no fetal cell detection in maternal blood of women with younger sons (Bianchi *et al.*, 1996; Abbud Filho *et al.*, 2006).

**Figure 1:** Microchimeric fetal cells present in the appendix of pregnant women. (A and B) H&E staining of appendix tissue. (A) Different layers of the appendix (2× magnification): L, lumen; M, mucosa; SM, submucosa; IC, inner circular smooth muscle; OL, outer longitudinal smooth muscle; S, serosa. (B) IC with neutrophilic infiltration (arrows) (60× magnification). (C and D) Male cells identified by XY-FISH at the SM (C) and IC layers (D). Male cells with X and Y chromosomes labelled with SpectrumOrange™ and SpectrumGreen™, respectively (arrows). (E and F) Male cells identified by Y-FISH at the SM (E) and IC layers (F). Male cells with Y chromosome labelled with SpectrumOrange™ (arrows). (G and H) Combined Y-FISH and immunohistochemistry of the SM (G) and IC layers (H). (G) CD3+ Y+ lymphocyte. (H) Desmin+ Y+ muscle cell. 100× magnification (except for A and B).
2002). Our results in appendices removed during pregnancy support this and suggest that the number of fetal microchimeric cells is lower during pregnancy but increases during the years after pregnancy, possibly due to local proliferation of fetal microchimeric cells with multi-lineage capacity.

Although small study numbers may have limited statistical analysis, our results appear to show that two factors may affect the frequency of FCMC: the reproductive history and the degree of tissue inflammation. Women with current male pregnancies (A3, A5, A6, A7, A8) tend to have higher rates of microchimerism than those with previous male pregnancies (A2, A4, A9). Surprisingly, patient A4, with no proven previous male pregnancy, had more microchimeric cells in the appendix than patients A9 and A2, who both had had sons 2 years before the index female pregnancy. According to Bianchi et al. (2001), women with a history of fetal loss exhibit a high frequency of FCMC (Bianchi et al., 2001). This may explain the slightly higher rate of microchimerism of patient A4 compared with A9 and A2, as a spontaneous abortion of unknown gender was reported in her reproductive history. The frequency of microchimerism also seems related to the tissue histology. We observed that women with tissues proved to be inflamed by the time of appendicectomy had more microchimeric male cells than those with non-inflamed appendices. Artlett (2005) hypothesized that fetal cell response in maternal organs might be specific to different types of injury. It has also been suggested that fetal cells invade the affected maternal tissues when a certain threshold of damage is reached (Khosrotehrani and Bianchi, 2005), which could be what had happened in the appendix, as patients with more severe injuries (acute appendicitis) had higher microchimeric frequencies. However, as male cells were also detected in the specimens with mild inflammation, it seems that these tissues do not need to reach a certain threshold for microchimeric cells to be present. While we acknowledge limitations of small numbers/limited histologies, our work suggests that fetal cells respond to tissue injury according to the type of injury and mainly in proportion to the degree of damage.

An association between FCMC and maternal tissue repair in humans has already been reported. Srivatsa et al. (2001) identified male cells in thyroid specimens with the appearance of normal thyroid follicular cells. Similarly, in this study, we identified in injured tissues male cells morphologically identical to the surrounding population of cells, but showed that some male cells had differentiated into muscle cells (desmin⁺) and T-lymphocytes (CD3⁺). These findings suggest that fetal cells of mesenchymal and haematopoietic lineages have the ability to engraft within the muscle and mucosa/submucosa layers of the appendix during pregnancy. We speculate that once present in the peripheral blood, fetal cells may be randomly distributed to different maternal organs (including the appendix), where they engraft and may persist for decades, as suggested in previous studies (Bianchi et al., 1996; O’Donoghue et al., 2004). In the presence of inflammation, and in response to the signals produced by damaged tissues (Yen et al., 2006), engrafted fetal cells may have the capacity to proliferate locally and differentiate into specialized cells such as muscle and lymphocytes. Alternatively, and considering our previous work, fetal cells transferred into maternal blood may engraft in the maternal bone marrow (acting as a long-term reservoir of stem cells), and upon tissue injury may migrate to sites of injury and become involved in tissue repair (O’Donoghue et al., 2004,2008). Mesenchymal cells are one of the fetal cell types implicated (O’Donoghue et al., 2003,2004), in which light we have recently shown that fetal mesenchymal cells preferentially home to sites of tissue injury in fetal transplantation models (Chan et al., 2007; Guillot et al., 2008a,b). However, due to methodological limitations, it is not possible to address the origin of fetal microchimeric cells in humans. Immunohistochemistry allows identification of the type of microchimeric cells, but not their origin.

In this study, we used stringent measures to minimize contamination to avoid false positive results and controls without sons were consistently negative. Analysis of tissue was blinded and independently verified. For the detection of male cells we primarily relied on FISH, which permits quantification of fetal cell number, localization and morphology (Choolani et al., 2001; O’Donoghue et al., 2004). Thus, we first used the DYZ1 probe labelled with SpectrumGreen™ for the quantification of microchimeric fetal cells in the appendix and then used the same DYZ1 probe labelled with SpectrumOrange™ both to confirm the results and to perform combined immunohistochemistry and FISH. Male cells in female tissues could originate from other sources; however, we confirmed that none of the women had twin pregnancies, or history of blood transfusion or transplantation. Despite identifying desmin⁺ and CD3⁺ male cells in the maternal appendix, we were unable to determine whether microchimeric cells were actively involved in tissue repair or simply a marker of inflammation. We believe that desmin⁺ and CD3⁺ male cells are more than tissue markers, considering that we identified male CD3⁺ cells in tissues where, according to the histopathological analysis, the inflammatory process was reported to have already resolved (A8—resolving appendicitis). Therefore, male fetal CD3⁺ (and possibly male desmin⁺) cells may be involved in tissue repair/regeneration, i.e. they may take part in the repopulation of appendix cells after the inflammation had led to cell death. Male CD3⁺ cells found in the appendix may be actively involved in the inflammatory process, as these are presumed to be activated T-lymphocytes, but given the limitations of working on archived specimens, as well as the complexities of T-cell function, it is not possible to address the exact role of these cells from this work.

It is important to note that our findings are preliminary and need confirmation with a larger number of samples and more varied clinical histories. Further studies are needed to address whether fetal cells actively participate in tissue repair. Murine models circumvent many of the limitations of human studies, allowing tracking of fetal cells during and after pregnancy, full characterization of fetal cell types in the mother and determination of whether fetal cells result in functional improvement in response to maternal injury (Bianchi, 2007).

In conclusion, we identified male cells of putative fetal origin in the appendix tissues of pregnant women who had...
undergone appendicectomy. Microchimeric cells were evenly distributed in the muscle, mucosa and submucosa layers of the appendix tissue. Data obtained from morphology and co-localization analysis, as well as from combined immunostaining and Y-FISH, suggested that microchimeric cells present in the appendix of pregnant women were able to differentiate into muscle (desmin+) and lymphocytes (CD3+). We conclude therefore that during pregnancy fetal cells are recruited to sites of injury, where they proliferate locally, and speculate they may be stem cells or cells with multi-lineage capacity, of either mesenchymal or haematopoietic origin. When present at the sites of injury, such cells may actively participate in tissue repair.

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