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Preparation of granulocyte concentrates by apheresis: collection modalities in the USA

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A previous International Forum (IF) addressed collection modalities of granulocytes (i.e. neutrophils) for supportive therapy (*Vox Sang* 2010; 88:567–575).

Contributions to this IF came only from European centres because physicians representing centres from the United States of America (USA) elected not to participate. However, after publication of the IF, it was suggested by Professor Strauss that other centres from the USA be invited to describe their collection modalities, because it was anticipated that there might be noteworthy differences in practices between centres in Europe and the USA. Indeed, some differences have now become apparent, based on responses from four different centres in the USA.

Question 1

Donor selection

- Do you collect granulocytes exclusively from your pool of healthy blood donors or do you also include the patients' relatives as donors?
- When selecting the donor, do you predominantly select CMV-negative donors for CMV-negative recipients?

Question 2

Mobilization regimen

- Does your national law allow the administration of G-CSF to unrelated donors
- Does your national law allow the administration of G-CSF to related donors
- Which dose of G-CSF is administered to the donors and at how many hours before donation?
- Do you combine G-CSF with steroids?
 - If yes, at which dose and which kind of steroids – prednisolone or dexamethason (DXM)?
- If steroids are given alone, at how many hours before collection?
 - prednisolone (dosage)?
 - DXM (dosage)?

Question 3

Frequency of donations

- Do you restrict the frequency of donations if the donor has been primed with G-CSF, steroids or both?
- What are your regulations concerning the accumulation of HES in the donor?

Question 4

Sedimentation agents

High- (Hetastarch, HS) and low molecular weight hydroxyethyl starch (Pentastarch PS) are used as sedimentation agents to enhance granulocyte collection.

- Which kind of hydroxyethyl starch is used at your institution for granulocyte collection?

Question 5

Compatibility tests

- Do you respect or ignore the ABO and Rhesus match between donor and recipient?
- Do D-negative women younger than 50 years receive granulocytes only from D-negative donors?

Question 6

Screening for leucocyte antibodies

Because of the risk of TRALI, the ISBT working party has recommended to screen donor and recipient for the leucocyte antibodies. In case of positivity of either donor or recipient, a crossmatch between the plasma/serum of the positive individual and the leucocytes of the negative individual was recommended [4].

- Do you screen or crossmatch the donor and recipient for leucocyte antibodies (HLA and HNA)?
- If positive, which consequences are drawn?
- Do you routinely reduce the plasma donations from female donors?

In the USA, G-CSF is not approved by the FDA to be given to apheresis donors for mobilizing granulocytes. Furthermore, 'granulocytes apheresis' are not listed as an FDA-licensed product. In three of the four responding USA centres, the dose of G-CSF given to all donors before each collection is a standard one of 480 µg, whereas it is 600 µg in the fourth centre. In all centres, G-CSF is combined with oral dexamethasone (8 mg). In contrast, six European centres give varying doses of G-CSF according to the donors' body weight, and only two apply a standardized dose of 300 µg G-CSF to all donors. Further, some European centres use only steroids for donor stimulation, while others use G-CSF alone. Although steroids alone are used by some of the other centres in the USA, they are not used alone by the four responding centres because granulocyte yields are substantially lower than those when donors are stimulated with combined G-CSF + dexamethasone. Moreover, the frequency of donations per donor is significantly less frequent in Europe than in the USA.

Another difference is that in the USA, high molecular weight HES is used in three of the four centres because granulocyte yields are superior when compared to lower molecular weight HES, whereas this higher molecular weight HES is applied in only three European centres because of preference in Europe for the lower molecular weight formulation of HES.

In two of the three USA centres that answered the question concerning ABO and Rh-D compatibility, ABO compatibility is required – similarly to Europe, where it is required in all but one centre. In two of the four USA centres, Rh-D compatibility is required. Screening donors for HLA and/or HNA antibodies is rarely performed, either in the USA or Europe.

The apparent variation in methods for collecting granulocyte concentrates by apheresis requires interested readers to study all available reports and then to select the procedure that best suits their practice. This challenging field is certainly open for further research, and this IF may serve as a stimulating basis for further investigations.

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Question 1

Donor selection

- Do you collect granulocytes exclusively from your pool of healthy donors or do you also include the patients' relatives as donors?

Our primary approach is to access the patient's relatives and his/her social network, which may include fellow parishioners, co-workers and friends. When donors cannot be accessed through the patient's social network, we call in volunteers from our pool of platelet apheresis donors to provide the granulocyte concentrates.

- When selecting the donor, do you predominantly select CMV-negative donors for CMV-negative recipients?

CMV status of the donor and recipient is not a consideration in the donor selection process. We do not test the donors for CMV; thus, our patients receive granulocyte transfusions with an unknown CMV status.

Narvios A, Pena E, Han XY, Lichtiger B: Cytomegalovirus infection in cancer patients receiving granulocyte transfusions. *Blood* 2002; 99:390–391

Martinez F, Sazama K, Narvios A, Lichtiger B: Outcome of transfusion of CMV sero-negative immunosuppressed cancer patients with CMV sero-positive granulocytes. *American Journal of Clinical Pathology* 2004; 122:461

Question 2

Mobilization regimen

- Does your national law allow the administration of G-CSF to unrelated donors?
- Does your national law allow the administration of G-CSF to related donors?
- Which dose of G-CSF is administered to the donors and at how many hours before donation?
- Do you combine G-CSF with steroids?

Our national law allows the administration of G-CSF to related and unrelated donors only for stem-cell donors, but we use it 'off-label' for granulocyte donors, as do many others in the USA. We prescribe 600 µg (micrograms) of G-CSF as a single dose together with 8 mg of dexamethasone tablets (p.o.) 12 h before the procedure. The medications are dispensed to the donor at the Donor Center after he/she submits a blood sample for West Nile testing. The medications are self-administered around 8:00 PM, and granulocytes are collected at 8:00 AM the following day.

The premedication protocol we follow is based on the following reference:

Jendiroba DB, Lichtiger B, Anaissie E, Reddy V, O'Brien S, Kantarjian H, Freireich EJ: Evaluation and comparison of three mobilization methods for the collection of granulocytes. *Transfusion* 1998; 38:722–728

Question 3

Frequency of donations

- Do you restrict the frequency of donations if the donor has been primed with G-CSF, steroids or both?

All granulocyte donor candidates must be available for a platelet apheresis donation. In this fashion, we can assess each donor's venous access capabilities, evaluate his/her infectious disease testing results and, in general, determine whether the donor has the stamina and clinical condition to undergo a 2- to 3-h procedure with two needles in the antecubital veins. Frequently, we can determine that donors either do not have the venous structure to complete the procedure, do not realize the potential time commitment (as some change their

minds during the platelet apheresis procedure) or are ineligible to participate because of their infectious disease testing results.

Once the donor has demonstrated that he/she can tolerate the apheresis procedure, we proceed with premedication the following day, after evaluating all test results and determining that the donor meets all the criteria for successful granulocyte collection.

We collect granulocytes from related and unrelated donors every other day according to the premedication protocol described earlier after assuring that the CBC is within normal ranges. However, in general, patients do have extended social networks with several eligible donors, and we collect granulocytes from each donor every week or every other week, as deemed necessary by the patient's condition.

- What are your regulations concerning the accumulation of HES in the donor?

We monitor the donors for weight gain and evidence of fluid overload.

Question 4

Sedimentation agents

- Which kind of hydroxyethyl starch is used at your institution for granulocyte collection?

We use 6% hetastarch in 0.9% sodium chloride.

Question 5

Compatibility tests

- Do you respect or ignore the ABO and Rhesus match between donor and recipient?

We ignore the ABO and Rhesus match between donor and recipient when the donor is ABO incompatible and Rh incompatible. We do, however, drain by gravity the RBCs in such cases.

Narvios AB, Reddy V, Lichtiger B: Method of removing incompatible red blood cells from granulocyte components. *Transfusion and Apheresis Science* 2006; 35:179–180

Question 6

Screening for leucocyte antibodies

- Do you screen or crossmatch the donor and recipient for leucocyte antibodies (HLA and HNA)?

We currently do not screen donors for HLA or HNA antibodies; however, when we did screen donors in the past for HLA, it was not a definitive parameter in deciding on granulocyte infusion.

- If positive, which consequences are drawn?

When we did screen for HLA, we infused granulocytes from patients with HLA antibodies without any adverse effects. (Manuscript in preparation).

- Do you routinely reduce the plasma in donations from female donors?

We do not reduce the plasma in donations from female donors; furthermore, we accept female donors regardless of their number of pregnancies.

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Question 1

Donor selection

- Do you collect granulocytes exclusively from your pool of healthy blood donors or do you also include patients' relatives as donors?
- When selecting the donor, do you predominantly select CMV-negative donors for CMV-negative recipients?
- The NIH Clinical Center Department of Transfusion Medicine collects granulocyte concentrates from healthy volunteer HLA-typed cytopheresis donors. Most are long-time repeat donors recruited from a cohort of plateletpheresis donors. Patients' relatives are accepted if they meet the criteria established for other allogeneic donors and if there is no medical contraindication. Our granulocyte donors are not screened for anti-CMV. CMV serostatus may change, and a requirement for repeat testing would prohibit timely apheresis donation. CMV-non-tested donors are used to support CMV-negative patients if the medical benefit appears to outweigh the risk.

Question 2

Mobilization regimen

- Does your national law allow G-CSF to unrelated donors?
- Does your national law allow G-CSF to related donors?
- Which dose of G-CSF is administered to the donors and how many hours before donation?

G-CSF (filgrastim) is not licensed by the FDA for mobilizing granulocytes. Therefore, all donors at NIH, related and unrelated, must be admitted to an IRB-approved protocol and give written consent as part of their predonation evaluation prior to receiving G-CSF. Granulocyte concentrates are not labelled as FDA-licensed products.

The G-CSF dose is 480 µg given as a single subcutaneous dose 12–20 h prior to donation. G-CSF is supplied by the manufacturer as a 1.6-ml single-use vial containing 480 µg. Approximately 70% of our donors experience adverse effects at this dose of G-CSF. Symptoms include bone pain, headache, fatigue, insomnia and flushing and respond readily to ibuprofen or acetaminophen. Donors are advised to take these medications every 4–6 h as needed.

- Do you combine G-CSF with steroids?

We believe that if granulocytes are collected, it is imperative that a therapeutic dose be achieved. To maximize granulocyte numbers, all granulocyte donors receive a preparative regimen consisting of a combination of dexamethasone and G-CSF. A single dose of G-CSF increases the circulating neutrophil count by about 500%, and the combination of a single

dose of G-CSF plus dexamethasone increases the neutrophil count by 700%. The granulocyte content of the product increases correspondingly by 300–400% (1).

Donors intolerant to dexamethasone and those who prefer not to receive this agent may be stimulated with G-CSF alone.

- If yes, which dose and which kind of steroids – prednisolone or dexamethasone (DXM)?

The dexamethasone dose is 8 mg (supplied as two 4-mg tablets) taken orally 1 h after dinner of the evening prior to donation, approximately 12 h prior to the procedure. As our unpublished experience (in press) suggests an increased risk of posterior subcapsular cataracts in donors who are repeatedly stimulated with steroids, all donors receiving dexamethasone undergo a comprehensive eye examination at least every 2 years.

- If steroids are given alone, at how many hours before collection?

We discourage the use of dexamethasone alone, because the granulocyte dose collected is often marginal. Stimulation with dexamethasone alone is permitted only for small recipients (< 20 kg) and then only with specific exception by the physician in charge.

- DXM dosage?

The dexamethasone dose is 8 mg orally one hour after dinner on the evening prior to donation, approximately 12 h prior to the procedure.

Question 3

Frequency of donations

- Do you restrict the frequency of donations if the donor has been primed with G-CSF, steroids or both?

Granulocyte donations are generally permitted no more frequently than once per month; the interval is arbitrary. Increased frequency and decreased intervals between mobilizations are considered if the donor is an HLA match for an alloimmunized recipient. In our published experience, the long-term (10 years) effects of repeated G-CSF stimulation in 83 unrelated volunteer granulocyte donors who received G-CSF three or more times between 1994 and 2002 were no different than in repeat plateletpheresis donation [2]. Although the number of granulocyte donors studied is small and continued surveillance of healthy individuals after G-CSF is prudent, our data suggest that G-CSF/dexamethasone stimulation is safe.

- What are your regulations concerning the accumulation of HES in the donor?

There are no national regulations or standards in the U.S. regarding accumulation of HES. We limit the volume of HES infused to 500 ml per granulocytapheresis donation, not to exceed 12 donations per year.

Question 4

Sedimentation agents—High- (Hetastarch) and low molecular weight HES (Pentastarch) are used as sedimentation agents to enhance granulocyte collection

- Which kind of HES is used at your institution for granulocyte collection?

We use the high molecular weight sedimenting agent Hetastarch (mean molecular weight 600 kDa, molar ratio 0.75) based on our own published studies [3]. We prospectively compared the granulocyte collection efficiency, granulocyte yield, and adverse reaction rate in 72 randomly paired (hetastarch versus pentastarch) granulocytapheresis procedures from 36 volunteer donors. In 33 of 36 donors, hetastarch procedures were significantly more

efficient and yielded more granulocytes. No starch-induced reactions were seen with either agent. We have no evidence of long-term complications [2]. We believe that the benefit of collecting more granulocytes outweighs the potential disadvantage of less rapid starch elimination.

Question 5

Compatibility tests

- Do you respect or ignore the ABO and rhesus match between donor and recipient?
- Do D-negative women younger than 50 years receive granulocytes only from D-negative donors?

We respect both the ABO and Rh match, however neither precludes the use of a mismatched donor if the potential medical benefit exceeds the risk. For major ABO incompatibility where granulocyte transfusion is considered vital, we decrease the red cell content of the component by sedimentation [4]. More than 90% of the red cells can be removed efficiently with recovery of ~80% of granulocytes; the residual red cell contamination is about 3 ml. Using this technique, we observed no transfusion reactions or signs of hemolysis after transfusion of 66 ABO-incompatible granulocyte concentrates [4].

Whereas we prefer D(−) components for D(−) recipients, we have transfused D(+) sedimented, red cell reduced granulocytes followed by intravenous RhIg when necessary to prevent alloimmunization [5]. Two patients with chronic granulomatous disease were administered 32 and 13 daily granulocyte transfusions, 18 and 2 of which, respectively, were D(+). After the first dose of intravenous RhIG, both patients exhibited (passive) circulating anti-D that was undetectable 3–4 years later. Similarly, two patients with severe aplastic anemia were given 5 and 14 granulocyte transfusions, 4 and 7 of which, respectively, were D(+). Our experience suggests that intravenous RhIG can be used to reduce or prevent alloimmunization to D in D(−) patients receiving sedimented, red cell reduced D(+) granulocyte transfusions [5].

Question 6

Screening for leukocyte antibodies—Because of the risk of TRALI, the ISBT working party has recommended to screen donor and recipient for the leukocyte antibodies. In case of positivity of either donor or recipient a crossmatch between the plasma/serum of the positive individual and the leukocytes of the negative individual was recommended.

- Do you screen of crossmatch the donor and recipient for leukocyte antibodies (HLA or HNA)?
- If positive, which consequences are drawn?
- Do you routinely reduce the plasma donations from female donors?

Our volunteer granulocyte donors are all HLA-typed and we screen prospective granulocyte recipients for HLA antibodies. Because granulocyte increments and circulation are poor, and because alloimmunized patients often react to granulocyte transfusions, we try to avoid administering granulocytes to alloimmunized patients if the donor expresses the cognate antigen [6]. Patients with chronic granulomatous disease, who are otherwise immunocompetent, may become immunized during a lengthy course of granulocyte transfusions, so we screen for HLA antibodies periodically during transfusions, after adverse reactions, or before subsequent courses of transfusion. If HLA antibodies are present, we discourage further granulocyte transfusions unless the granulocytes are collected from HLA-compatible donors [6]. Although reactions to transfusion are more common, the post-transfusion neutrophil increment may not be significantly affected by HLA

alloimmunization. In thirty-two patients with severe aplastic anemia who underwent granulocyte transfusions, no difference in mean post-transfusion absolute neutrophil count was noted between the patient groups divided according to HLA alloimmunization status; we thus do not consider HLA alloimmunization an absolute contraindication to granulocyte therapy [7]. We advise close and careful monitoring of HLA-alloimmunized patients receiving granulocytes. We do not screen granulocyte donors for HLA or HNA antibodies, and do not routinely screen recipients for HNA antibodies.

We do not screen granulocyte donors for the presence of high-titer ABO isohemagglutinins. We do not routinely reduce the plasma content from female donors.

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T. H. Price

Question 1

Donor selection—Do you collect granulocytes exclusively from your pool of healthy blood donors or do you also include the patients' relatives as donors?

Almost all granulocytes are collected from our pool of healthy donors, the donors that also routinely donate platelets. On occasion, we will collect granulocytes from donors who are the patient's friends or family members.

When selecting the donor, do you predominantly select CMV-negative donors for CMV-negative recipients?

Yes, CMV sero-negative donors are always used for patients who are CMV sero-negative. We also currently use only CMV sero-negative donors for patients who have undergone a cord blood transplant, regardless of the CMV status of the patient or the cord donor.

Question 2

Mobilization regimen—Does your national law allow the administration of G-CSF to unrelated donors?

Yes. G-CSF is a licensed drug in the United States. Although it is not licensed for this specific use, off-label use of licensed drugs is permitted by US law.

Does your national law allow the administration of G-CSF to related donors?

Yes. G-CSF is a licensed drug in the United States. Although it is not licensed for this specific use, off-label use of licensed drugs is permitted by US law.

Which dose of G-CSF is administered to the donors and at how many hours before donation?

At our institution, patients may be participating in a randomized controlled trial to assess the efficacy of high dose granulocyte transfusion therapy. If the patient is enrolled in this study, the donors are stimulated with G-CSF. If the patient is not participating in this study, G-CSF is not administered to the donor.

If G-CSF is administered, the dose is 480 mg, ideally administered 12 h prior to donation, almost always between 8 and 16 h prior to donation.

Do you combine G-CSF with steroids?

Yes.

If yes, at which dose and which kind of steroids – prednisolone or dexamethasone?

Dexamethasone, 8 mg.

If steroids are given alone, how many hours before collection are they given? Prednisolone (dosage)?

Dexamethasone (dosage)?

Ideally, 12 h prior to donation, almost always between 8 and 16 h prior to donation.

Question 3

Frequency of donations—Do you restrict the frequency of donations if the donor has been primed with G-CSF, steroids or both?

Yes. We limit the number of G-CSF-stimulated donations to 8 per year. This number is admittedly not evidence based. If the donor is stimulated with steroids alone, we limit the number of donations to 12 per year.

What are your regulations concerning the accumulation of HES in the donor?

There are no federal regulations regarding the accumulation of HES in the donors. AABB Standards require that each institution has a policy regarding limits on HES administration, and Standards limit the number of collections to a maximum of 24 per year. The limit of 24 collections per year is not specifically related to a concern about HES.

Question 4

Sedimentation agents—Which kind of hydroxyethyl starch is used at your institution for granulocyte collection?

We use Hetastarch.

Question 5

Compatibility tests—Do you respect or ignore the ABO and Rhesus match between donor and recipient?

We respect ABO/Rh. Donor and patient must be red cell compatible. A current red cell crossmatch (actual or electronic) is required. If a Group O donor is selected for a Group A or AB patient, the concentrate is plasma-reduced. No other combinations are plasma-reduced.

Do D-negative women younger than 50 years receive granulocytes only from D-negative donors?

In general, yes. Under unusual circumstances, granulocytes from Rh-positive donors may be provided for Rh-negative patients with the concurrence of the patient's physician. In these situations, the patient's underlying condition is such that subsequent pregnancies are considered highly unlikely.

Question 6

Screening for leucocyte antibodies—Do you screen or crossmatch the donor and recipient for leukocyte antibodies (HLA and HNA)?

We do not routinely screen for HLA sensitization, and we never screen for HNA sensitization. If the patient is known to be highly HLA sensitized (e.g. requiring HLA-matched platelets), we may try to provide HLA-selected granulocyte donors.

If positive, what consequences are drawn?

Laboratory or clinical evidence of HLA sensitization is taken into consideration but does not preclude the administration of HLA-unselected granulocytes. Additional care is taken to administer the granulocytes slowly and to carefully observe the patient for any evidence of a transfusion reaction.

Do you routinely reduce the plasma in donations from female donors?

No.

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Question 1: donor selection

- With rare exception, granulocytes/neutrophils are collected from our pool of healthy apheresis donors – with the only considerations for donor–recipient matching being RBC compatibility between recipient and donor and being certain that recipients who need cytomegalovirus (CMV) ‘safe’ components receive them from CMV antibody–negative donors. The rare exception, when a relative donor might be selected, is when the patient is receiving ‘matched’ platelet transfusions because of refractoriness, and the relative granulocyte/neutrophil donor is one of the matched platelet donors. Many years ago at our hospital, bone marrow transplant recipients received post-transplant prophylactic granulocytes/neutrophils from their marrow donors – most of whom were relatives[1].
- Yes, recipients needing CMV safe products nearly always receive granulocytes/neutrophils from CMV antibody–negative donors. Rarely, a CMV antibody–negative recipient, who is being transplanted with stem/progenitor cells from a CMV antibody–positive donor, may receive CMV antibody–positive granulocytes/neutrophils when they are needed urgently and no CMV antibody–negative donors are available.

Question 2: mobilization regimen

- Although the USA Federal Food & Drug Administration has not approved G-CSF to be used for granulocyte/neutrophil donor stimulation (regardless of whether donors are related or unrelated to the recipient), G-CSF stimulation is commonly used in the USA – with written, informed consent of the donor. We inject one vial (480 µg) subcutaneously as close as possible to 12 h before beginning leukapheresis. To deal realistically with donor and blood centre time schedules, G-CSF can be injected within a ‘window of time’ 8–16 h before leukapheresis begins (i.e. 12 ± 4 h).
- G-CSF is combined with dexamethasone, which is given as 8 mg orally at the time of the G-CSF injection. We never use only corticosteroids for donor stimulation because the number of granulocytes/neutrophils collected is quite inferior to combined G-CSF and dexamethasone mobilization.

It is important to note that a concern has been raised over the possible increased risk of posterior subcapsular cataracts in granulocyte/neutrophil donors because of repeated corticosteroid administration [2,3]. Because the exact clinical importance of this finding is still unclear and because granulocyte/neutrophil yields are superior to combined G-CSF and dexamethasone (over G-CSF alone) mobilization, we continue to use both mobilizing agents. However, consideration must be given to educating donors about this possible risk during the informed consent process.

Question 3: frequency of donating

- Donors are restricted to donating granulocytes/neutrophils no more often than once every 72 h and no more than eight times per 12 months.
- Donors may receive no more than one litre of either 6% hetastarch or 10% pentaspan solution per each donation – thus, no more than one litre every 72 h and/or no more than eight litres every 12 months.

Question 4: sedimentation agents

- Although we were one of the blood centres involved in studying pentastarch as a replacement for hetastarch during leukapheresis for granulocyte/neutrophil collection [4], we now use only hetastarch because leucocyte yields are much higher with current preparations of hetastarch. Clearly, hetastarch persists in donor blood stream longer than pentaspan [5] and has more profound effects on plasma-clotting factors [6], but with the limitations on doses and frequency of donation, risks to healthy donors are minimal [7].

Question 5: compatibility tests

- Yes, we respect recipient–donor ABO and Rh(D) antigen compatibility. As an exception, Rh(D)-negative men and post-menopausal women may receive granulocytes/neutrophils from Rh(D)-positive donors – if the recipient is not already alloimmunized to Rh(D) antigen and if no Rh(D)-negative donors are available.

Question 6: screening for leucocyte antibodies

- Neither donors nor recipients are screened for leucocyte antibodies. Currently, we have no restrictions on the use of women for apheresis donations (i.e. either platelets or granulocytes/neutrophils), but in the future, we may consider using only women, who have never been pregnant or performing leucocyte antibody testing to exclude antibody-positive donors. Only one-third of our women apheresis donors have not been pregnant. Apheresis components from women donors are not volume-reduced to remove part of the plasma.
- Common sense dictates that, if multiple granulocyte/neutrophil donors are available for any given recipient, the donor of choice would be a man followed by a woman with no known history of pregnancy.

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