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Abstract: Maternal immunization offers much hope to substantially reduce morbidity and mortality from infectious diseases after birth. The success of tetanus, influenza and pertussis immunization during pregnancy has led to consideration of additional maternal immunization strategies to prevent Group B Streptococcus (GBS) and respiratory syncytial virus (RSV) infections, among others. However, there remain multiple gaps in our knowledge regarding the immunobiology of maternal immunization that prevent optimal design and application of this successful public health intervention. An innovative landscape analysis was therefore undertaken to identify research priorities. Key topics were delineated through review of the published literature, consultation with vaccine developers and regulatory agencies, and a collaborative workshop gathering experts across several current maternal immunization initiatives - GBS, RSV, pertussis, and influenza. Finally, a global online survey prioritized the identified knowledge gaps based on expert opinion regarding their importance and relevance. This article presents the results of this worldwide landscape analysis and discusses the identified research gaps.

Maternal Immunization: Collaborating with Mother Nature

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Summary

Maternal immunization offers much hope to substantially reduce morbidity and mortality from infectious diseases after birth. The success of tetanus, influenza and pertussis immunization during pregnancy has led to consideration of additional maternal immunization strategies to prevent Group B Streptococcus (GBS) and respiratory syncytial virus (RSV) infections, among others. However, there remain multiple gaps in our knowledge regarding the immunobiology of maternal immunization that prevent optimal design and application of this successful public health intervention. An innovative landscape analysis was therefore undertaken to identify research priorities. Key topics were delineated through review of the published literature, consultation with vaccine developers and regulatory agencies, and a collaborative workshop gathering experts across several current maternal immunization initiatives - GBS, RSV, pertussis, and influenza. Finally, a global online survey prioritized the identified knowledge gaps based on expert opinion regarding their importance and relevance. This article presents the results of this worldwide landscape analysis and discusses the identified research gaps.

1 **Introduction**

2 Failure to improve survival in neonates by 2035 from the current status is estimated to lead to
3 116 million preventable stillbirths or neonatal deaths, 99 million survivors with disability,
4 and millions more with a lifelong increased risk for non-communicable diseases (1). The
5 underlying causes for the 2.6 million stillbirths per year are largely unknown, but
6 approximately 20% of the 2.9 million annual neonatal deaths are thought to be due to
7 infection (1). The transfer of antibodies from pregnant women to their offspring is profoundly
8 important for the health and survival of neonates and young infants, in particular by reducing
9 the risk of severe infections. Unfortunately, not all pregnant women have protective levels of
10 antibodies against pathogens affecting their offspring. The strategy of immunizing pregnant
11 women to enhance protection of young infants is rapidly gaining support from both the public
12 and health professionals alike (2). Contributors to this momentum include the global
13 reduction in neonatal tetanus as a result of maternal immunization, the benefits of seasonal
14 and pandemic influenza immunization for both mother and infant, and the positive impact of
15 immunization during pregnancy on recent pertussis outbreaks. These results are also
16 stimulating commercial development of new vaccines against additional threats such as group
17 B Streptococcus (GBS) and respiratory syncytial virus (RSV).

18 Recognizing the need to enhance the science of maternal immunization, the Bill and Melinda
19 Gates Foundation (BMGF) commissioned the authors to conduct a landscape analysis of the
20 immunobiology underpinning successful vaccination during pregnancy. The scope of the
21 review included all relevant immunobiological issues in general terms and as applied to
22 immunization against pertussis, influenza, GBS, and RSV specifically. The analysis also
23 aimed to identify differences that might be encountered among pregnant women in low and

1 middle income countries (LMICs) compared with high income countries (HICs) that may
2 affect the success of maternal immunization programs. An innovative approach was used to
3 rapidly identify and prioritize the current knowledge gaps in order to inform future studies.
4 This article describes the methodology and the results of this effort and discusses the
5 identified research gaps in immunobiology of maternal immunization that are generalizable
6 across pathogens. The research gaps specific to individual pathogens are discussed in two
7 companion articles. Other crucially important aspects of maternal immunization—safety,
8 public perception, and integration into existing global immunization programs—are outside
9 the scope of the project and will not be discussed here but are discussed in recent publication
10 summarizing the outcome of a series of meetings sponsored by the National Institute of
11 Health (3).

13 **Landscape Review Process and Knowledge Gap Prioritization**

14 To best capture the current state of knowledge, an innovative multi-stage review process was
15 undertaken. A detailed description of the methodology used and of the results of the analysis
16 is provided as Supplemental Materials. Briefly, an international team of 10 recognized
17 experts undertook a scoping review of the published English language literature since 2000.
18 The experts summarized the state of knowledge pertaining to their assigned area, including
19 their assessments of the gaps in understanding the biology of the immunization processes.
20 The team met at a collaborative workshop in Vancouver to share their assessments with 26
21 additional international experts who commented critically on the presentations (videos from
22 this meeting are available upon request from corresponding authors). Over 100 knowledge
23 gaps were identified through this process, attesting to the under-development of the

underlying science. To ensure that sufficiently broad deliberation was achieved and issues affecting translation addressed, further consultations were held with leaders of maternal vaccine development programs at 3 major vaccine companies and representatives of 2 major regulatory agencies (the US Food and Drug Administration and the European Medicines Agency) who freely shared their insights into the knowledge gaps and challenges.

To prioritize the identified knowledge gaps, topics considered most relevant during the collaborative workshop were included in an online survey completed by nearly 200 “content experts” from the global maternal immunization community. Respondents rated the importance of each knowledge gap; the results were remarkably consistent among respondents, including industry representatives, academic researchers, and national immunization policy makers. The top 20 knowledge gaps are listed in Table 1; each was rated ≥ 4 out of 5 (high to very high importance). To prepare the present and companion reviews, the authors integrated and summarized the information gathered from each of the above steps.

General Considerations Regarding Maternal Immunization Strategies

When considering the 4 disease targets for maternal immunization included in the landscape analysis, it is striking that no two are alike (Table 2), and that different strategies will likely be needed for each disease. All of which may make the production of a combined vaccine challenging. In order to focus on the immunobiology of maternal immunization, contextual differences, such as maternal disease risk, infant disease burden, global epidemiology, and microbial diversity will not be discussed further in this article. The common goal among maternal vaccination programs is temporary protection of the young infant against severe

1 illness and death by ensuring sufficient and timely transfer of protective antibodies from the
2 mother. This passive protection should persist until the infant is no longer at a high risk of
3 diseases (e.g. until 3 months of age for GBS disease) or until protection can be achieved by
4 active infant immunization (e.g. pertussis). Protection of the infant may also be achieved
5 indirectly by reducing carriage and/or disease in the mother, which subsequently reduces
6 transmission of pathogens to the infant (e.g. GBS, pertussis). Whether or not protection of the
7 mother against disease is also required is another important factor in determining the timing
8 of maternal immunization. In the case of influenza, for example, it may be that immunization
9 early during pregnancy would be favoured to protect both the pregnant woman and neonate.
10 Finally, there may be additional benefits of pre-pregnancy immunization, to prevent
11 infections which may have harmful effects on the developing fetus. It is important to note that
12 a substantial limitation in our understanding of optimal maternal immunization for any target
13 is the lack of defined correlates of protection for young infants. Without a validated measure
14 of protection it will be difficult to compare results of studies in different settings or to
15 improve vaccines or immunization regimens using serologic criteria.

16 Immunization during pregnancy relies on the capacity of the pregnant woman to mount
17 appropriate primary or secondary antibody responses, depending on whether the pathogen has
18 been encountered prior to pregnancy. The notion that pregnancy is associated with the
19 induction of a number of immunoregulatory mechanisms that are essential for the survival of
20 the fetus suggests that antibody responses to vaccines may be different in pregnant compared
21 with non-pregnant women. Vaccine responses may be further influenced by complications
22 affecting pregnant women, such as chronic infections. Optimal protection of the young infant
23 is considered to rely on the effective transfer of maternal immunity through the placenta and

the persistence of this passive immunity for the duration of infant exposure to the particular pathogen. Additional protection may be provided by transfer of immunity via breast milk. However, the relative contributions of breast milk and serum antibodies to infant protection will be difficult to define but important to understand, especially for infants born prematurely with limited transplacental transfer of antibodies. These passively transferred maternal immune factors can further influence active immunity induced in the infant by natural infection or immunization. Sixty-eight knowledge gaps with regards to the impact of pregnancy on vaccine responses, the transfer of maternal immunity to the infant, and on infant immunity were identified following the collaborative workshop (Supplemental Material). The top 10 of these knowledge gaps were considered most relevant in the on-line survey are presented in Table 1.

Impact of pregnancy on vaccine responses

Studies indicate that pregnancy influences B cells and antigen-presenting cells (APCs); the potential impact on follicular helper T cells has not been assessed at all.

Pregnancy and B lymphocytes

Estrogen and pregnancy reduce B cell lymphopoiesis in mice (4). Reduction in circulating B cells numbers have also been shown in pregnant women but the potential impact on antibody responses to primary immunization is unknown (5–7). Few studies have suggested an impact of pregnancy on memory B cell subsets but no consistent picture has yet emerged (8–10). In addition, the potential impact of pregnancy on other B cell subsets, including transitional or marginal zone B cells, remains to be assessed. In populations living in LMICs, chronic exposure to microbial antigens such as *Plasmodium falciparum* induces high frequencies of

1 circulating atypical memory B cells (8,9). As these memory cells have a reduced capacity to
2 produce immunoglobulins, their increased frequency may limit responses to recall
3 immunization in both pregnant and non-pregnant individuals living in LMICs.

4 ***Pregnancy and immunoglobulins***

5 Studies regarding the influence of hormones on B cell functions support the notion that
6 pregnancy may impact the production of immunoglobulins. Estrogen increases the production
7 of IgG by human B cells (11). In addition, activated human B cells upregulate the expression
8 of the prolactin receptor and prolactin further decreases the threshold of B cell activation
9 (12). In mice, estrogen also upregulates the expression of the activation-induced deaminase,
10 the enzyme that initiates somatic hypermutation and class switch recombination of
11 immunoglobulins (13). On the other hand, serum IgG levels have been found to be lower in
12 pregnant than in non-pregnant women in both LMIC and HIC settings (14,15). The
13 mechanism involved is unclear, but could, at least partly, be due to hemodilution. Pregnancy
14 is also associated with modifications in IgG glycosylation(16). IgG are glycoproteins
15 carrying N-glycans at both the Fc and Fab segments which modulate their effector functions
16 (17). In pregnancy, total IgG have increased sialylation and decreased N-acetylglucosamine
17 bisection of both Fc and Fab fragments and increased galactosylation of Fc fragments (16).
18 Although the functional consequences of Fab fragment glycosylation remain unclear,
19 sialylation and galactosylation of Fc fragments have been associated with decreased
20 inflammation and were suggested to be involved in the remission of rheumatoid arthritis
21 associated with pregnancy (18,19). The potential implications of the anti-inflammatory
22 properties of maternal IgG on immune homeostasis and anti-microbial defenses in the fetus
23 and newborn have not been determined. Surprisingly, IgG of different antigen specificity

1 have different glycosylation profiles and this profile is modified following recent antigen
2 exposure (20). Moreover, IgG glycosylation patterns are different in populations living in
3 HICs versus LMICs (20). Studies are needed to determine the impact of pregnancy on the
4 glycosylation and effector functions of vaccine-induced IgG.

5 ***Pregnancy and antigen-presenting cells***

6 Pregnancy is associated with changes in numbers and phenotype of APCs. The number of
7 myeloid dendritic cells (mDCs) increases in the first trimester of pregnancy and decreases as
8 pregnancy progresses to reach similar counts in the third trimester as in non-pregnant women
9 (21,22). On the other hand, the number of plasmacytoid (pDCs) is reduced during the third
10 trimester of pregnancy (23). mDC and pDC were shown to express higher levels of Toll-like
11 receptors in pregnant compared with non-pregnant women (24). A number of differences
12 exist between APC from females and males that are induced by sex hormones and could
13 therefore be relevant to pregnancy (25). Modifications of APC are likely to be important for
14 successful pregnancy but the potential impact on vaccine responses have not been
15 determined.

16 ***Pregnancy and vaccine response***

17 The impact of pregnancy and sex hormones on B cells and APC suggests a possible influence
18 on antibody responses to vaccines. This potential is indirectly supported by the observation
19 that the magnitude of antibody responses to many vaccines is often higher in females than in
20 males (25). Most studies of pregnant women that demonstrated potent vaccine
21 immunogenicity, however, did not include a comparison with non-pregnant women (26–29).
22 Few controlled studies have been conducted that generally involved only small study
23 populations. Some studies reported similar responses to seasonal influenza vaccines in

1 pregnant and non-pregnant women whereas others detected differences in titers or
2 seroconversion rates (30–34). Factors responsible for the discrepancies between studies may
3 include differences in tested vaccines and participant characteristics. Two controlled studies
4 conducted in HICs showed similar antibody responses to Tdap immunization in pregnant and
5 non-pregnant women while two other studies in LMICs reported no impact of pregnancy on
6 the response to tetanus immunization (35–38). The immunogenicity of a conjugated GBS
7 vaccine was recently studied in South Africa (39). Although the responses were not compared
8 between pregnant and non-pregnant women, the vaccine was immunogenic in both. Whether
9 the gestational stage of pregnancy affects responses to vaccines has not been extensively
10 studied. Similar antibody responses to seasonal and pandemic influenza vaccination were
11 observed throughout pregnancy in two studies while a trend towards higher seroconversion
12 rates with a seasonal influenza vaccine was seen during the third trimester in one study
13 (27,31,40). The impact of pregnancy on the quality of antibody response to vaccines remains
14 largely uncharacterized. Conflicting results on the avidity of antibodies following pertussis
15 immunization during early compared with late in pregnancy have been obtained in relatively
16 small scale studies (41,42).

17 The persistence of antibodies following maternal immunization will influence the optimal
18 timing of immunization and the requirement to repeat immunization during consecutive
19 pregnancies; however, relatively little information on this topic is available. Antibody decay
20 following immunization with adjuvanted pandemic influenza vaccine was similar in pregnant
21 and non-pregnant women (33). Pertussis immunization is currently recommended during the
22 second or early third trimester of pregnancy to achieve sufficiently high titers of antibodies
23 close to delivery (<http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6207a4.htm>). This

1 recommendation is challenged by a recent study showing higher titers of cord blood
2 antibodies following pertussis immunization during the second compared with the third
3 trimester of pregnancy, suggesting cumulative transfer of antibodies (43).

4 Innate immune responses following maternal immunization have not been explored. One
5 study reported similar plasma levels of inflammatory cytokines in pregnant and non-pregnant
6 women following seasonal influenza immunization. This is in line with the similar or even
7 lower reactogenicity observed in pregnant women following influenza immunization (44,45).

9 ***Influence of maternal factors on vaccine responses***

10 Most studies reported no significant effect of maternal age, parity, socioeconomic status or
11 body weight on antibody response to vaccines during pregnancy (46–48). But parity was
12 associated with reduced antibody responses to *H. influenzae* type b conjugate vaccine in The
13 Gambia and with higher responses to pertussis toxin in Belgium (49,50). This finding may be
14 particularly important in LMICs where high order multiparity is more common. Few studies
15 suggested a limited impact of nutrition on vaccine responses during pregnancy (51,52).
16 Whether obesity affects immune response to vaccination in pregnancy is poorly understood
17 as very obese women (BMI >30) are typically excluded from clinical trials. Relatively little
18 information is available regarding the possible differences in vaccine immunogenicity
19 between LMIC and HIC resulting from health conditions of the mother. One study reported
20 no impact of *P. falciparum* parasitemia at the time of immunization on antibody response to
21 tetanus toxoid (35). However, HIV infection impairs responses to vaccines. In South Africa,
22 pregnant women with HIV infection have lower seroconversion rates after seasonal influenza
23 vaccination compared with uninfected pregnant women but antibody half live and vaccine

efficacy are comparable between the two groups (53,54). HIV infection was also associated with lower immunogenicity of a glycoconjugate GBS vaccine in pregnant women in South Africa (55). The impact of helminth infection on vaccine responses during pregnancy has also not been systematically analyzed (56).

Summary

Overall, studies indicate that antibody responses to recall immunization are comparable between pregnant and non-pregnant women. Whether primary responses to new vaccines will be impacted by pregnancy is still unknown. Limited data suggest that pregnancy might impact avidity maturation, class switch, and glycosylation of vaccine-induced antibodies. With the exception of HIV infection, maternal factors influencing responses to vaccines have not been clearly identified.

Transfer of maternal immunity through the placenta

IgG transfer and preterm birth

IgG is the only antibody which is directly transferred across the placenta (57). Recent studies indicate that other maternal Ig can be transported to the fetus when complexed with IgG (58). IgG are actively transported through the placenta by the neonatal Fc receptor (FcRn), and possibly by additional receptors that have not yet been identified (59,60). The FcRn is expressed by syncytiotrophoblasts covering the surface of the chorionic villi and transports IgG by transcytosis into the fetal circulation. Although the FcRn is expressed and functional in the placenta from the first trimester, most of the antibody transfer occurs after 28 weeks gestation (61,62). Preterm birth is therefore an important factor limiting the transfer of

maternal immunity through the placenta and may affect the transport of IgG1 more than IgG2 (63–66).

Preterm birth occurs in 5% to 18% of pregnancies globally and is a leading contributor to infant morbidity and mortality. In a recent systematic analysis, over 60% of all preterm births were estimated to occur in sub-Saharan Africa and South Asia (over 9 million of approximately 15 million births per year globally) (67). At 28–33 weeks gestation, fetal-maternal antibody ratios are typically 0.5–0.6, compared with ≥ 1.0 at term. Thus transfer of maternal antibody could therefore afford some potential protection even in prematurely born newborns if their levels were elevated by prior immunization (66).

Factors influencing IgG transfer

The rate of IgG transfer through the placenta is influenced by several factors including IgG subclass, antigen-specificity, and chronic maternal infections. IgG subclasses are transcytosed at different rates, with IgG1 being most actively transferred, followed by IgG4, IgG3, and IgG2 (59,68,69). IgG3 allotypes have different affinity for FcRn and this results in differential transfer ratios (69). It is puzzling that antibodies of different antigen specificities are transported at different rates across the placenta, resulting in different maternal:cord blood antibody ratios (70–72). Reported cord blood:maternal ratios range as high as 1.9 for pertussis to as low as 0.7 for GBS, with influenza ranging from between at 0.7 to 1.0 depending on the study (26,53,73–75). These differences may be partly related to the differences in IgG subclass proportions, as protein antigens generally induce IgG1 and IgG3 subclasses while polysaccharide antigens induce mainly IgG2 antibodies, but this hypothesis has not been systematically examined (57,72). Whether or not the structure of maternal IgG influences placental transfer beyond subclasses has not been clearly established. Few studies

1 have suggested that high avidity antibodies may be transferred preferentially across the
2 placenta (76,77). Historical studies also suggested a preferential transfer of
3 hypergalactosylated IgG but this notion is not supported by a more recent study based on
4 more advanced technologies showing no impact of Fc galactosylation on transfer (78,79).
5 Chronic maternal infections and hypergammaglobulinemia have a profound impact on
6 maternal antibody transfer (66). Reduced transfer of IgG is observed in women with
7 hypergammaglobulinemia, a phenomenon that may be related to the saturation of FcRn (80–
8 82). Hypergammaglobulinemia and the denudation of syncytiotrophoblasts from chorionic
9 villi could also be involved in the reduced transfer of IgG associated with placental malaria
10 (66,81). A recent study in Papua New Guinea indicated an association between reduced
11 transfer of respiratory syncytial virus (RSV)-specific IgG with hypergammaglobulinemia but
12 not with placental malaria itself (83). Maternal HIV infection also results in a reduction of
13 maternal IgG transfer (82,84–86). Intriguingly, the impact of chronic maternal infections and
14 hypergammaglobulinemia appear to depend on the subclass and antigen-specificity of IgG. In
15 a study in South Africa, maternal HIV infection was associated with reduced transfer of
16 naturally acquired GBS-specific IgG1 but not IgG2 (85). In a study in The Gambia, maternal
17 hypergammaglobulinemia was found to be associated with impaired transfer of total IgG1
18 and IgG2, but not IgG3 and IgG4, and with a reduced transfer of IgG against pathogen but
19 not vaccine antigens (81).

20 **Summary**

21 Transfer of maternal antibodies through the placenta mostly occurs after 28 weeks gestation
22 and is limited by preterm delivery and by chronic maternal infections. Maternal
23 immunization could compensate for this reduced transfer but the timing of maternal

immunization and vaccine formulations will have to be optimized to achieve this objective. The basis for the variable maternal antibody transfer according to their antigen specificity remains poorly understood. Further studies are needed to determine the role of IgG subclass or other structural characteristics in this variability in maternal transport.

Transfer of maternal immunity through breastfeeding

The importance of breast milk in post-natal life is highlighted by the strong correlation between breastfeeding and the profound reduction of risks of infection and infection-related mortality in infancy (87,88). However, only one study assessed the role of breastfeeding in protection against an infectious pathogen following maternal immunization. In Bangladesh, exclusive breastfeeding was associated with fewer episodes of respiratory illness with fever in children born to mothers immunized against influenza during pregnancy (89). Prevention of infectious diseases by breastfeeding is thought to be due to the strengthening of gastrointestinal and respiratory mucosal immunity by: (1) improving the function of the epithelial barrier through breastmilk high content of growth factors; (2) transferring antimicrobial factors such as lactoferrin and lysozyme; and (3) transferring microbial antigen-specific immunity (Figure 1). Maternal immunization may thus modulate antigen-specific immune factors in breast milk and promote antigen-specific immune responses in infants.

Breast milk IgA

Breast milk secretory IgA (sIgA) antibodies are specific for an array of common intestinal and respiratory pathogens because the selective migration of B cells originating from the mucosal membranes to the mammary gland (90). Higher levels of sIgA should therefore be induced by mucosal as compared with systemic immunization, as observed following HIV

immunization of lactating Rhesus macaques (91). The antimicrobial properties of sIgA depend on: (1) the inhibition of pathogen adherence to and invasion of mucosal epithelia; (2) the neutralization of pathogens and toxins; (3) the transfer of antigens across the mucosal barrier and the stimulation of low level inflammation (reviewed in (92)). The latter mechanism has been mainly described in mice. Few studies in humans have demonstrated the transport of milk IgA into the circulation of breastfed mature and premature newborns (90,93,94). In LMIC where prematurity and gut mucosal inflammation are frequent, IgA transport to neonatal circulation may be increased and prolonged and could therefore be particularly beneficial. On the other hand, breast milk IgA may have a negative impact on the response to mucosal vaccines, but this finding remains controversial (95,96).

A number of studies showed increased levels of antigen-specific IgA in breast milk following maternal immunization against influenza, pertusis, RSV, *Streptococcus pneumoniae* and *Neisseria meningitidis* (reviewed in (97)). The amount of breast milk and magnitude of secretory IgA responses against a consensus HIV envelope protein were recently associated with the reduced risk of postnatal transmission of HIV in Malawi. This observation highlights the need for development of maternal vaccination strategies increasing HIV-1 envelope-specific breast milk IgA to reduce mother-to-child HIV transmission (98). Importantly, maternal conditions that are known to negatively impact transplacental transfer of IgG do not affect IgA transfer through breast milk. Prematurity increases the transfer of growth and immune factors, particularly IgA, in colostrum and milk (99,100). Furthermore, breast milk concentration of total and pathogen-specific IgA is not affected by maternal HIV infection or by malnutrition (101–104).

Breast milk IgG

Breast milk IgG originate from serum through FcRn transport and from milk resident B lymphocytes (105). Total breast milk IgG concentration is about 10% of IgA concentration but it tends to increase with duration of breastfeeding (100,106,107). Increased concentrations of antigen-specific IgG are detected in breast milk following immunization against RSV and pneumococcus and following natural infection with GBS, rotavirus, and HIV (96,108,109). Evidence of a protective role of breast milk IgG was demonstrated in studies on HIV infection, where IgG had higher neutralizing activity than IgA, mediated antibody-dependent cellular cytotoxicity, and were inversely correlated with the risk of HIV transmission (109). Breast milk IgG were also inversely correlated with cytomegalovirus (HCMV) load, suggesting a protective role against HCMV transmission (110). However, the role of breast milk IgG in the defense against other pathogens has not been studied. Mouse experiments indicate that breast milk IgG can cross the gut barrier through FcRn and can thereby promote the transport of IgG-antigen immune complexes and stimulate immune response to antigens and pathogens (60,111–114). Whether this process occurs in humans is unknown.

Breast milk leucocytes

Breast milk contains neutrophils, macrophages, and lymphocytes (115). Common infections increase the number of total leucocytes in breast milk but whether similar changes occur post-immunization is unknown (116). Breast milk B lymphocytes are IgG producing memory cells. Their antigen-specificity was demonstrated in the context of HIV infection (105). Similarly, HIV-specific CD4 and CD8 T lymphocytes were detected in breast milk and may contribute to virus control through inflammatory cytokines and cytotoxicity (117,118).

1 Studies suggest that breast milk CD4 T cells may be transferred to human neonates and
2 induce transient specific cellular immunity (93,119,120).

3 ***Transfer of microbial antigens through breast milk***

4 Although pathogens can be detected in breast milk following maternal infection, transmission
5 to the offspring is not commonly observed, with notable exceptions including HIV, HCMV,
6 and HTLV-1 (121). The evidence suggests that breast milk immunity may prevent pathogen
7 transmission. In addition, studies indicate that exposure to pathogens through breast milk
8 induces immune responses in infants independently of transmission. Exposure to HIV-
9 containing breast milk is associated with the induction of mucosal IgG and IgA responses and
10 with systemic cell-mediated immune responses in uninfected infants (102,122). Similarly,
11 *Vibrio cholera* can be transferred through breast milk and induce either disease or
12 colonization associated with specific IgG responses in infants (123). These observations
13 suggest that breastfeeding can promote immunity to pathogens in infants by transmitting
14 pathogens that are attenuated by maternal immune responses and/or transfer of pathogen
15 antigens. Studies indicate that a similar process occurs following immunization of lactating
16 women with the live attenuated rubella vaccine (reviewed in (124)). Mouse studies have
17 shown that the intrinsic adjuvant properties of antigens, the level of IgG and vitamin A in
18 breast milk are critical factors in the induction of effector immune responses in the offspring
19 (125).

20 ***Summary***

21 There is strong evidence that breast milk is essential for mucosal immunity in infants and that
22 maternal vaccination increases antigen-specific immune effectors in breast milk. Mouse and
23 human studies further suggest that the transfer of microbes through breast milk may promote

active immunization in infants. Breast milk transfer of immunity by immunized mothers may be particularly relevant in LMIC where transplacental transfer of immunity is reduced by chronic maternal infections and the high rate of pre-term delivery. However, there currently exists little data linking breast milk immunity induced by vaccines and infant protection.

Maternal immunization and infant immunity

Following transfer across the placenta, maternal antibodies are expected to protect the infant from disease. However, a certain level of antibody (the presumed correlate of protection) has to be reached to provide clinical protection and this level needs to be maintained until the infant is no longer at risk, or is protected by active immunization. How long maternal antibodies persist above the protective levels in the infant is a function of the concentration of the antibody in the newborn at birth and the antibody half-life ($T_{1/2}$). Thus, the transplacental transfer and decay kinetics of maternal IgG in the infant are key determinants of the duration of protection. However, high levels of maternal antibodies present at the time of infant vaccination may also interfere with the immune response of the infant to the respective vaccine. Lastly, maternal immunization can have effects on the fetus and newborn infant beyond passive protection.

Prevention of infection and disease

The distribution of serum antibodies beyond the bloodstream of the neonate/infant is not well defined, but could limit what is achievable in terms of mucosal protection. For example, very little IgG is detectable in saliva of young infants until the teeth erupt (126), making sterilizing immunity against respiratory pathogens unlikely. A more readily achievable objective would then be the minimization of invasive disease severity rather than prevention of portal of entry

infection/colonization. This limitation is illustrated by the failure of various preparations of pertussis immune globulin to prevent colonization (and subsequent invasive infection) in humans and animal models (127–129). The recently observed effectiveness of maternal pertussis immunization in preventing infant disease represents an important advancement (130). If the benefit is largely attributable to minimization of disease severity such encounters could result in passive-active immunity, with active immunity following attenuated natural infection (131).

Maternal antibody decay in infants

The $T_{1/2}$ of IgG differs by subclass and is not a fixed entity but is directly proportional to the total IgG concentration; this is called the *concentration-catabolism effect*, where IgG catabolism is accelerated in subjects with increased IgG levels and conversely, reduced in subjects with a low serum IgG concentration (132). The molecular mechanisms underlying the differences in $T_{1/2}$ of the various IgG subclasses as well as the concentration-catabolism effect center around FcRn (59,60). Subclass and structural modifications of IgG have profound impact on the interaction with FcRn, and thus $T_{1/2}$. For example, IgG3 allotypes have different affinity for the FcRn and this results in different $T_{1/2}$ (69). Also, aglycosylated human IgG1 has a significantly shorter $T_{1/2}$ (62 h) than the glycosylated form (153 h) (132). As indicated above, glycosylation of maternal antibodies is modified during pregnancy (16,133), but how this relates to $T_{1/2}$ in the infant is currently not known. Furthermore, studies suggest that the $T_{1/2}$ of IgG in infants varies depending on the antigen-specificity of the antibodies as well as between populations. For example, reported $T_{1/2}$ in the infant of maternal antibodies specific for pertussis antigens is ~30-40 days, for tetanus ~50 days, but for GBS ~60 days (29,134,135). $T_{1/2}$ of maternal antibodies of a given specificity can also

1 vary substantially between populations; whether this variability involves differences in IgG
2 subclass or other structural differences has not been delineated (136–138).

3 ***Interference with infant immunization***

4 The presence of maternal antibodies to a particular vaccine antigen has been reported to
5 reduce antibody generation following vaccination of the infant with the same antigen
6 (reviewed in (139–141)). This is called *interference*. Maternal antibodies not only affect
7 levels of antibodies produced by the infant, but also can influence their quality (strength of
8 antigen binding or avidity) (141,142). Priming of T cell responses to vaccines does not appear
9 to be affected by passive antibodies and this probably contributes to the good response to
10 booster doses (139,140). The key factors influencing interference are antigen-specific
11 maternal antibody titers at time of infant immunization, as well as infant vaccine antigen-
12 content (including dose). For pertussis, maternally derived antibodies have been shown to
13 interfere with antibody responses with whole-cell vaccines, but less so when acellular
14 vaccines were used in the infant (37,50,143–147). Whether the improved response to
15 acellular vs. whole-cell vaccine among those with higher antecedent PT titers is due to higher
16 antigen load in the acellular product or to the absence of other components of the whole cell
17 vaccine lacking in the acellular product has not been determined (148). Given that the current
18 lead candidates for a maternal GBS vaccine are TT- or CRM197-conjugate polysaccharide
19 vaccines, it is worth noting that infants born to mothers with high anti-TT titers immunized
20 with Hib-T-conjugates have reduced anti-GBS responses but infants immunized with HbOC
21 (CRM₁₉₇) showed no interference (149–151). Although several mechanisms have been
22 proposed, the molecular and cellular basis of the interference remains incompletely
23 understood (139,140).

Influence of maternal immunization on infant beyond passive immunity

Following influenza (TIV) vaccination during pregnancy, anti-HA and anti-matrix protein IgM antibodies could be detected in 38.5% and 40.0%, respectively, of cord blood specimens (152). Given that IgM does not cross the placenta, this would be indicative of an active adaptive B cell response in the fetus. This was further corroborated by the detection of HA-specific T cell responses in some newborns of immunized women using synthetic peptide-HLA multimers. Similarly, earlier studies of tetanus vaccination during pregnancy reported detection of anti-toxoid IgM in sera of some infants (153,154). Furthermore, given that vaccines can have immune modulatory effects in postnatal life beyond initiating antigen-specific adaptive responses, i.e. non-specific effects (NSE) (155), it is conceivable that immunization during pregnancy could also have NSE not only in the mother, but also in the fetus and/or newborn. To our knowledge, this has not been systematically investigated. However, MF59-adjuvanted influenza vaccination during pregnancy led to an altered cytokine production profile in the nasal mucosa of 4 week old infants contrasting infants from vaccinated vs. unvaccinated mothers (156). The clinical relevance of either of these ‘unexpected’ findings (active *in utero* immune response; non-specific effects on the newborn after maternal immunization) is currently not clear.

Summary

Immunobiological parameters such as correlates of protection based on passively acquired antibody levels and half-life of the antibody are key determinants of the efficacy of maternal immunization. However, little is known about either aspect. Higher maternal antibody levels in the infant can interfere with the infant’s response to immunization; neither the mechanisms involved nor the relevance of this for protection have been determined. Finally, maternal

immunization may also prime immune responses in the fetus and thereby influence responses after birth.

Concluding remarks

The passive transfer of maternal immunity is considered central to anti-microbial defenses in early life (Figure 2). The proposed mechanisms center around active transport of maternal IgG through the placenta providing systemic immunity during the first months after birth until the infant actively acquires immunity through exposure to pathogens or vaccines. The immune components of breast milk can provide longer-term immunity at the mucosal level and could also contribute to the development of infant immunity at the systemic level.

Although maternal immunization is an effective strategy to increase anti-microbial immunity in early life, many knowledge gaps remain in our understanding of vaccine responses during pregnancy, the transfer and persistence of maternal immunity in infants, and the interactions between maternal antibodies and the infant immune system. This landscape analysis prioritized gaps that are of particular relevance to the development of new vaccines for pregnant women and to the implementation of maternal immunization worldwide (Table 1). Filling those gaps offers the potential to further improve this important public health intervention. This will require immunological studies of existing vaccines administered to pregnant women and the inclusion of immunological endpoints in the clinical studies of vaccines that are under development.

Contributors Statement

AM, DWS and TRK developed and managed the landscape analysis and synthesized the information. AM, VV, LP and TRK led the literature review on the immunobiology of maternal immunization. MG and GB provided major administrative support and participated in the synthesis of the information. AM, MS, ND, VV, LP, CEJ, SAH, KME, PH, PO, DWS and TRK contributed to the literature review and synthesis. AM, MS, VV, MG, DWS and TRK drafted the initial manuscript and all authors contributed to the final version of the manuscript.

Declaration of interests

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Table 1. Global Experts Survey: Top 20 Knowledge Gaps

	Likert Rating score* (maximum score 5.0)
1. Immunization During Pregnancy	
a) Impact of the type of vaccine antigen on maternal responses	4.1
b) Impact of health conditions on maternal immune responses	4.2
2. Transplacental Transfer of Antibodies	
a) Impact of timing of vaccination during pregnancy on net transfer	4.4
b) Impact of antigen type on maternal responses and transferability	4.1
c) Impact of pregnancy complications on antibody transfer	4.0
3. Protection of fetus and newborn infant	
a) Impact of maternal immunization regimen on cord titers	4.3
b) Impact of maternal immunization regimen on infant responses	4.3
c) Clinical relevance of interference with active immunization	4.3
d) Impact of maternal antibodies on effector and memory B cell responses of infants	4.0
e) Modulation of breast milk immune components by immunization	4.2
4. Pertussis vaccination	
a) Correlates of protection against colonization, disease, death	4.4
b) Requirement for multiple pertussis antigens, role of P toxin	4.2
c) Reactogenicity of repeated doses of Tdap in sequential pregnancies	4.0
5. Group B streptococcal vaccine	
a) Correlates of protection against colonization, disease, outcomes	4.5
b) Serotype specific immunogenicity, transfer and protection	4.3
c) Impact of serotype on correlates of protection	4.0
d) Effect of carrier proteins on responses of infants to vaccination	4.0
6. Respiratory syncytial virus vaccine	
a) Correlates of protection against infant disease, death	4.6

b) Protection against lower respiratory infection, disease	4.6
c) Impact of pre-existing immunity on maternal responses	4.0

*Rating score 4 = high importance, 5 = very high importance, on a 5 point Likert scale

Table 2. Maternal Immunization Landscape: No Two Programs are Alike

Consideration	Pertussis	Influenza	GBS	RSV
Maternal disease risk	+	+++	++	+
Infant mortality	++	+	+++	++
Infant disease frequency	+ (cyclic ¹)	++	+	+++
Disease seasonality	✓	✓	✗	✓
Microbial diversity	+	++	++	+
Licensed vaccine available	✓	✓	✗	✗
Maternal booster response expected ²	✓	Quasi ³	Not assumed	✓
Passive protection of infant	✓	✓	✓	✓
Maternal:cord Ab ratio	1.1-1.9	0.7-1.0	0.7-0.8	1.0
Antibody half-life (days)	36-40	40-50	30-44	36-79
Infant vaccination	✓	≥6 months	✗	(✓) ⁴
Correlate of protection	✗	Quasi ⁵	✗	✗
Functional immunoassay	✗	✓	? ⁶	✓
Competing control option	✗	✗	✓ ⁷	✓ ⁸

¹Increased disease incidence usually occurs every 3-4 years

²Via previous vaccination and/or infection

³Prior vaccination and/or infection will lead to partial protection due to virus evolution

⁴Monoclonal antibody administered to high risk infants during RSV season

⁵Correlates of protection based on hemagglutinin inhibition assay or microneutralization titers have not been validated in young infants and are not based on maternal immunization

⁶Bacterial killing in an opsonophagocytic assay has been suggested as a possible correlate of protection

⁷Intrapartum antibiotic prophylaxis has reduced the incidence of early onset GBS neonatal sepsis

⁸Monoclonal antibodies administered to high risk infants during RSV season reduces rates of hospital admission

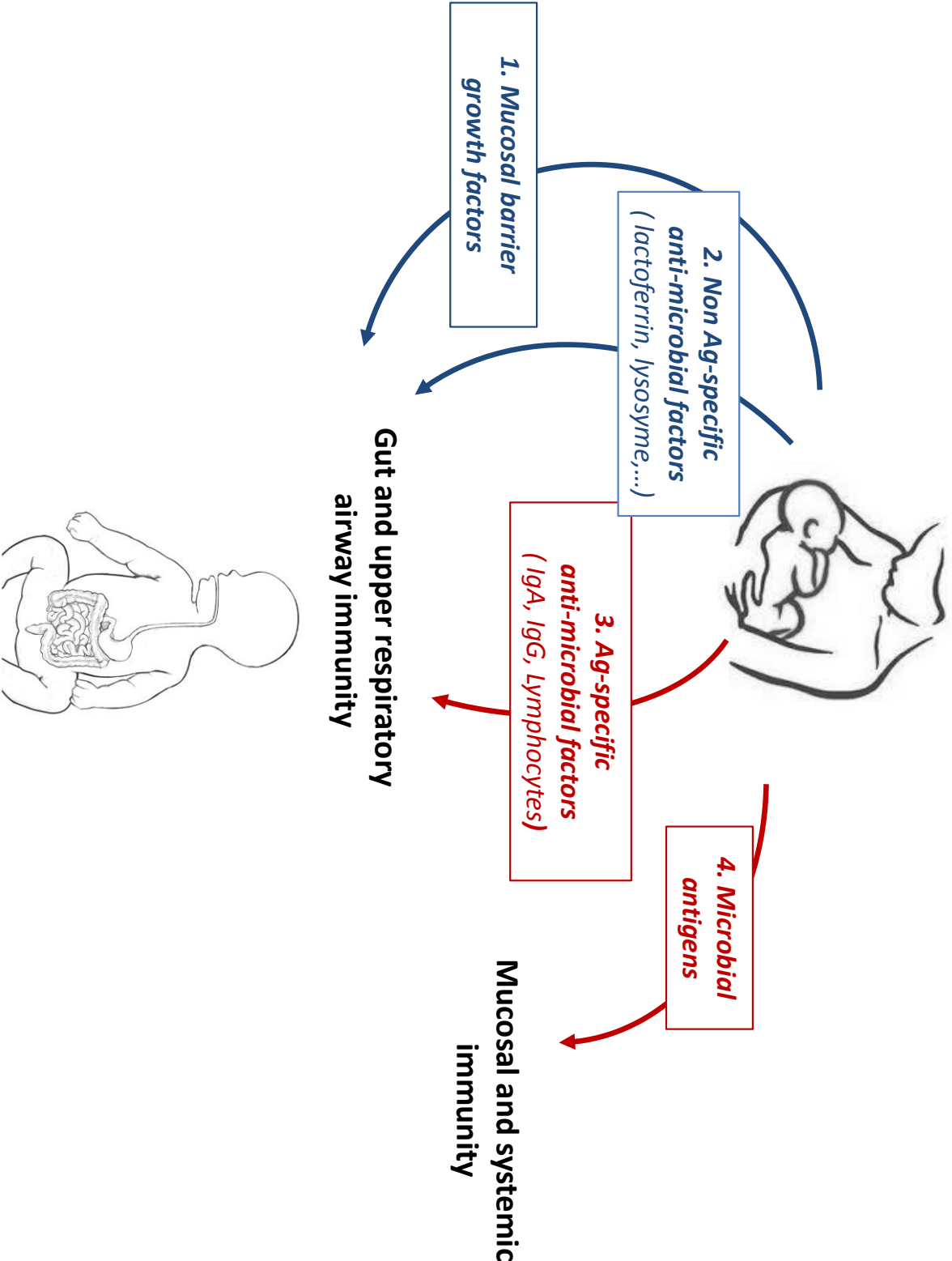


Figure 1. Transfer of maternal immunity through breastfeeding. Microbe-nonspecific immunity (blue) is promoted by breast milk through (1) growth factors improving the function of the epithelial barrier and (2) anti-microbial molecules. Microbe-specific immunity (red) is provided by Ag-specific maternal IgA, IgG and lymphocytes (3). Breast milk also contains antigens and/or attenuated microbes that may stimulate infant immunity (4). Maternal vaccination may improve prevention of infectious disease in breastfed children by increasing milk content in antigen specific anti-microbial factors and microbial antigens.

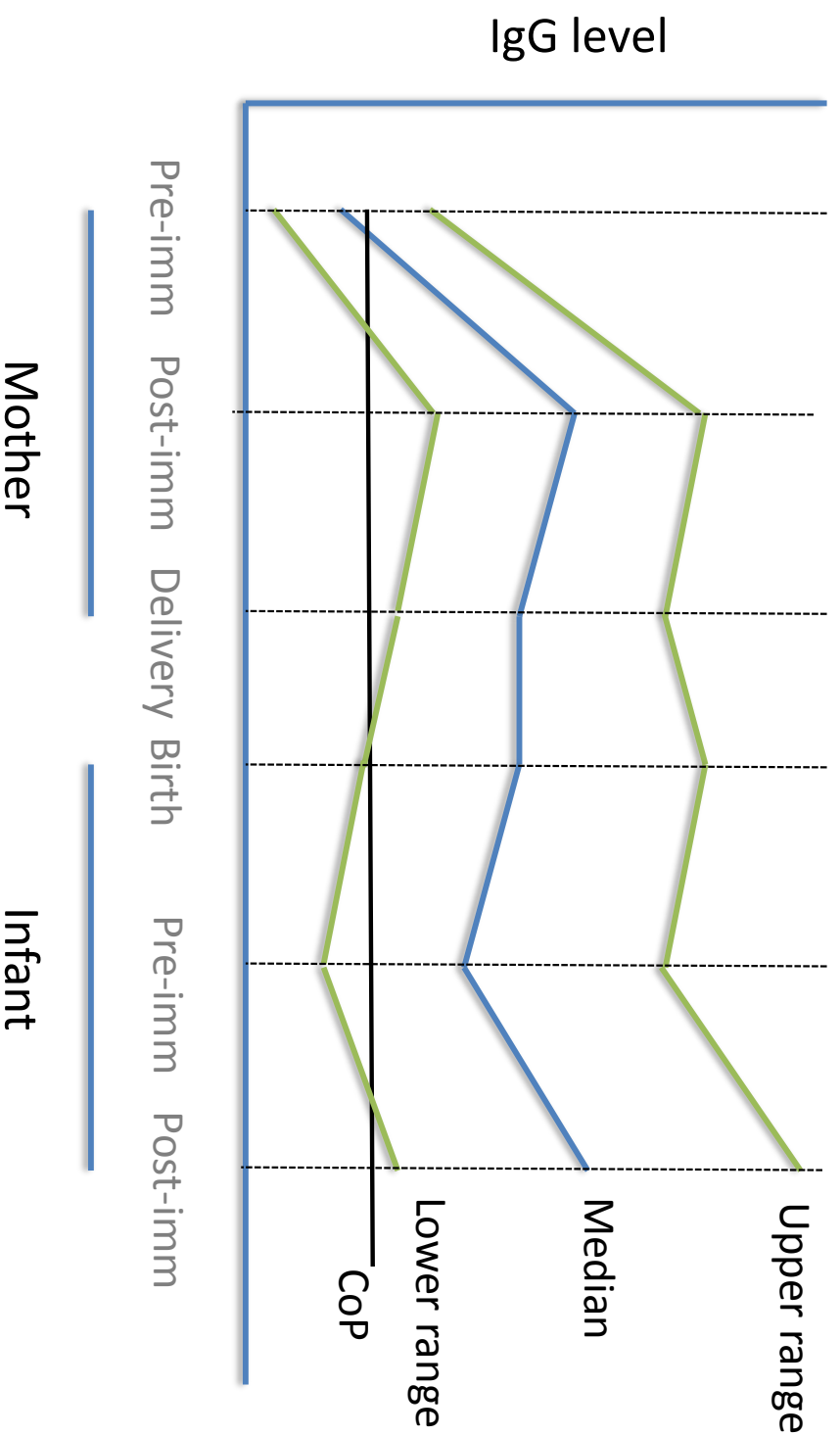


Figure 2. Influence of maternal immunization on infant IgG before and after vaccination. The horizontal black line represents a putative correlate of protection (COP) for the disease of interest. The blue line represents median of maternal immunization (Pre-imm), maternal IgG levels are low and may be below the COP. An ideal vaccine would raise this IgG level (Post-imm) such that even the lower end of the range would be above the COP, and would remain above the COP until delivery – this would depend upon the initial response to vaccination as well as timing between immunization and delivery. The infant IgG level at birth will depend on placental health, gestation and antibody-specific factors. This transferred maternal IgG level will fall until the infant receives additional protection through direct immunization, and the rate of fall will vary between pathogens and between individuals. Ideally, maternal vaccination would ensure the IgG level is above the COP until infant immunization, and this will be dependent on the initial IgG at birth and the interval until infant immunization (Pre-imm), and it may be that a ‘window of susceptibility’ is created when the IgG level is below the COP. Following infant immunization, the IgG level will rise again, and the extent of this would be influenced by any interference caused by the presence of maternal IgG.

Supplemental material.

An innovative approach to determine research priorities in maternal immunization through international collaboration

Supplemental Panel 1. Definitions

Planning team: The principal authors and an organizational team put in place to support the workshop and the online survey.

Domain/area: Gaps in maternal immunization research are broad and include general and disease-specific issues. We divided these issues into domains (also referred to as areas); e.g. pregnancy, neonates, or pertussis issues.

Domain/content experts: Contributing authors/experts specializing in one of the domains of research regarding maternal immunization.

Landscape analysis: The process of describing and interpreting the landscape of an area. Applied to our task ('determine research priorities in maternal immunization'), this process is to describe, classify and quantify the importance of knowledge gaps regarding the immune biology of maternal immunization as well as the network of cross-cutting themes connecting these knowledge gaps.

Scoping review: A scoping study (also referred here as "review") approach allow rapid mapping of concepts that support a research area (1); it gathers the main sources and types of evidence available. This differs from a *systematic review* where literature is identified, selected, and appraised with the goal of collecting and analyzing data from all studies on a given topic.

Attendees/participants: Recognized experts in various domains of research invited to the workshop. The participants played an essential role by providing critical opinions and perspectives on all data regarding maternal immunization.

Knowledge gaps: Insufficient evidence in an area of maternal immunization relevant to vaccine development and translation, including low and middle income country settings.

Survey: An online platform created for ranking the identified knowledge gaps to create an actionable short list.

The lead investigators (A.M. D.W.S., T.R.K.) enlisted an international team of domain experts (**Supplemental Panel 1**) to share the review tasks. This 10-member team designed and conducted the landscape analysis (**Supplemental Figure 1**), dividing it among themselves according to their area of expertise. This strategy allowed the review process to advance quickly despite the large number of publications to be reviewed. The individual experts had the advantage of substantial familiarity with their assigned areas, enabling rapid identification of the key literature. The immunobiology review was divided into several parts (Domain/area, see Supplemental Panel 1) as diverse expertise was required. Likewise, the reviews of pertussis, influenza, GBS and RSV vaccines were undertaken by individual domain experts, with help from local colleagues.

Overview

The first step consisted of a scoping review of the literature to evaluate current knowledge of the immunobiology of maternal immunization as well as the source and type of studies available. A written summary of the key findings of the scoping review was prepared by each domain expert. The reviews followed an agreed standard structure, which eased the synthesis of results and facilitated comparisons between the various areas of interest. Each contributor then presented their summary during a workshop held in Vancouver, Canada. The format of the workshop and the presentations allowed generous time for

discussions and questions to maximize input from additional expert delegates. Informed by contributions from the workshop attendees and prior consultations with industry and regulatory

Supplemental Panel 2. Criteria used to select articles for review.	
The following encompasses criteria used for all domain review, i.e.: Vaccinology and cross-talk, Breast milk, Placenta, Pregnancy, Neonates, GBS, Influenza, Pertussis, RSV.	
Study aim	
Study that evaluate the impact of a biological/immunological mechanism on maternal vaccination (domain specific)	
Type of article	
Original or Review articles	
Study population	
Humans (applied for initial search only)	
Date of publication (see note)	
Since 01-Jan-2000 until 01-March-2015 (applied for initial search only)	
Source of citation	
Identified via search as outlined in Appendix 1 for each domain	
Source of citation	
Relevant references identified in articles from original search	
Source of citation	
Known articles already contained within personal collection, or advised by other members of Consortium	
Language	
English	
Thesis	
PhD theses or other academic non peer-reviewed documents	
Note: Included articles since 1996 for GBS and articles since 1985 for Pertussis.	

agency representatives and the experts of the BMGF, the authors identified >100 research gaps. This attested to the lack of knowledge around the science of maternal immunization. However, the list needed to be shortened to be practical. Priority was placed on gaps that were deemed most relevant to advance vaccine development, including aspects key for effective maternal immunization programs in LMICs. In total, 45 knowledge gaps were selected for inclusion in an online survey completed by nearly 200 experts from around the globe. The survey ultimately identified 20 research gaps ranked as very/highly important.

Scoping review

A scoping study is a type of review used to “rapidly” map the key concepts of a research area and the main

sources and types of evidences available to support them (1). We utilized a scoping review to identify research gaps relating to the immunobiology of maternal immunization. This strategy was designed to identify all relevant sources of the published literature. Therefore, the initial search “terms/queries” did not contain strict limitations. Contrary to a formal literature review, the remainder of the scoping process was not linear but iterative, requiring thoughtful assessment by the domain expert at each stage. The experts reviewed published literature already available to them and extracted, from the references, related work that had not been known to them beforehand. Formal literature searches complemented and expanded the assessment of relevant literature and revealed what was missed or recent. Using this approach, the experts were able to rapidly assemble and assess the pertinent literature on which to base their individual summaries.

The following paragraphs describe the stages (or “steps”) for conducting a scoping review for the purpose of identifying research gaps: 1) decide on the broader question to be asked initially, 2) identify all relevant studies that fall into this broad topic, 3) select studies to include in a focused review, 4) record data about selected articles, and 5) summarize and report the results (1).

Our overarching research question was: What is known about the underlying biology and immunology impacting maternal immunization for prevention of infectious diseases in early life in general and relating to RSV, influenza, GBS, or pertussis in particular?

To identify relevant studies, each term in the question became a keyword and the source for relevant MeSH associations. Searches were performed using the following tools: Pubmed, Medline, Excerpta Medica dataBASE (EMBASE), Cumulative Index to Nursing and Allied Health Literature (CINAHL), and hand-searching of key journals, networks, organizations, and conferences. For reproducibility, the terms used in the various literature searches were recorded by each expert (**Supplemental Table 1**). As the searches were in progress, an exchange of search terms via a shared Dropbox (Dropbox.com, Dropbox Inc.) folder helped harmonize the process.

To select studies for their summary, each expert identified specific selection criteria. At the screening stage, selection was based on the expert's familiarity with the literature identified. A record of criteria used can be found in **Supplemental Panel 2** (the criteria were used for all domain review, i.e.: Vaccinology and cross-talk, Breast milk, Placenta, Pregnancy, Neonates, GBS, Influenza, Pertussis, and RSV). Articles of potential interest to a specific topic that were not accessible to the expert were recorded in a separate database. All the remaining articles were read in detail by each topic expert in order to make the final decision to include them in the review. The final article selection was made available to all authors via shared Dropbox folders. Results from the selection process, in terms of the number of articles remaining after each step, are shown in **Supplemental Table 2**. Detailed recording of each included article helped to summarize and categorize the articles and improved traceability and transparency of the review process. General and specific information was recorded for the final list of selected articles. We recorded the following parameters when they were available, relevant, or applicable: authors, year of publication, study location, population studied (mother during pregnancy, infants, etc...), type of study (randomized controlled trial, retrospective, etc...), bibliographic source, sample type, number of samples (N), aim of study, methodology, outcome measures, and key findings. Given the variety in publication styles and formats in addition to constraints in obtaining some of the information, it was sometimes not feasible to extract all information from all studies. Where applicable, categories were created to facilitate the dissection of the review process by each topic (**Supplemental Table 3**).

Expert reviews

Each expert summarized the data accumulated in the scoping review in a written report. These reports concluded with summaries of the key knowledge gaps as well as the domain expert's own recommendations on how to address the research gaps. To harmonize the reviews amongst the experts, we established a review template; this also facilitated the amalgamation of all the reviews into a final summary report. The efficiency of the review process also benefited from regular teleconference calls and emails among the experts, support teams, and the lead authors.

Each review included the following sections:

- *Introduction*: Placed the specific topic area in context and highlighted progress, challenges, and prospects.
- *Search*: Provided details of the literature search (such as **Supplemental Table 2**).
- *Results*: Divided according to categories created during the scoping process (such as **Supplemental Table 3**).

- *Summary of gaps:* Analyzed the data collected and identified under-represented or missing categories of research, type of research, or the extent of research evidence within a category.

Consultations and Workshop

To ensure that completed reports would include the views of key stakeholders outside of academia which included vaccine producers and vaccine regulators, the lead authors visited or interviewed project leaders at major vaccine companies active in this field. Additionally, they met with officials of regulatory agencies of the United States of America (Food and Drug Administration (FDA)) and the European Union (European Medicines Agency (EMA)), who have had direct experience with maternal immunization issues and programs. Each of these meetings presented an opportunity to explore knowledge gaps from different angles.

All 10 experts synthesized their key information in presentations to fellow authors and 26 invited international experts at a Consultative Workshop held in Vancouver, Canada in May 2015. The workshop planning committee strived to include several invited experts from each domain and across the spectrum of professional affiliations (academics, public health, etc.). Each author nominated invitees whose work featured prominently in their selected literature.

The consultative workshop participants were: Carol Baker, Houston, TX; Kang Chen, Ann Arbor, MI; James Crowe, Nashville, TN; Morven Edwards, Houston, TX; Adrian Erlebacher, New York, NY; Hayley Gans, Stanford, CA; Chrissie Jones, London, UK; Beate Kampman, The Gambia; Ruth Karron, Baltimore, MD; Mark Loeb, Hamilton, ON; Richard Lo-Man, Paris; Antoine Malek, Bern, Switzerland; Peter McIntyre, Sydney, AU; Kingston Mills, Dublin, Ireland; Thomas Moran, New York, NY; Flor Munoz, Houston, TX; Stefan Niewiesk, Columbus, OH; Marta Nunes, Johannesburg, S Africa; Sarah Rowland-Jones, Oxford, UK; Craig Rubens, Seattle, WA; Mark Steinhoff, Cincinnati, OH; Geeta Swamy, Durham, NC; Pierre Van Damme, Antwerp, Belgium; Marietta Vasquez, Guilford, CT; Sing Sing Way, Cincinnati, OH; Dapeng Zhou, Shanghai, China; Sharon Berquist, Peter Dull, Hani Kim, Lynda Stuart, Ajoke Ter Meulen, Niteen Wairagkar, Chris Wilson, Chris Karp, Keith Klugman, Bill and Melinda Gates Foundation, Seattle, WA.

Knowledge gaps and global survey

All research gaps identified during the workshop were noted. In total, 108 gaps were identified, attesting to the limited science underpinning maternal immunization. The full, unsorted list of gaps is shown in **Appendix 1**. From the full list of knowledge gaps, 45 were selected by attendees of the workshop for further critical appraisal by the global community of experts on maternal immunization. The gaps selected for inclusion in the survey were the ones likely to have more immediate impact on the development of vaccines and programs for maternal immunization and/or to represent key issues for lower and middle income countries. The 45 selected gaps are shown in **Supplemental Figure 2**.

An online survey was developed to prioritize the 45 selected gaps into a shorter, more actionable list. The survey was hosted by FluidSurveys at the University of British Columbia. This unique consultative process was intended to include most academic researchers who had published in the field in the last 5 years, as well as a wide range of industry experts and national immunization policy-makers. Expertise of invitees was wide-ranging and included immunology, vaccine trials, microbiology, epidemiology, and social sciences. Primary affiliations of invitees included universities, governments, industry, and non-government organizations (see demographics of

survey respondents in **Supplemental Table 4**). These individuals were approached via e-mail with a request to complete a confidential online questionnaire. The first survey invitation was sent on July 3rd, 2015; a second was sent between July 6th and 30th, 2015. At least two reminders were sent to non-responders at one week intervals. The survey closed in mid-August. For each of the 45 listed knowledge gaps, respondents were asked to rate the importance of the item using a 5-point Likert scale. Respondents could opt out of rating the importance of a gap if they lacked sufficient knowledge to do so. After rating the importance of a gap, respondents were asked to also rate the relevance of the item to each of the several considerations:

- *population diversity*:, i.e. maternal and infant variables (genetic, environmental, population health, etc) influencing responses
- *vaccine formulation*: including antigen choice, dosage, dosing schedule, etc
- *vaccine efficacy*: such as the effect of host variables on achievable protection
- *vaccine safety*: for both mother and infant
- *programmatic considerations*: such as factors affecting program delivery or acceptance rates

These ratings also used a 5-point Likert scale. Not all considerations were necessarily relevant to each survey item but listing all of them aided format consistency. Of the 410 experts reached by email, 194 (47%) submitted evaluable responses (an excellent response rate for a mid-summer survey of substantial length; median time of 22 minutes). Two-thirds indicated involvement in maternal immunization research within the previous 2 years (**Supplemental Table 4**). The 45 gaps were ranked in descending order of their rated importance. A number of gaps shared the same importance score in which case the ranking sequence was based on the order in which the item appeared in the survey (**Supplemental Figure 2**). The scores were calculated for all respondents and also compared between those with and without special expertise in that specific area. The results were remarkably consistent among respondents, including between respondents from industry and other backgrounds. Twenty knowledge gaps emerged as most important, all having mean scores between 4 and 5 (high to very high importance). These gaps are discussed in detail in the individual reviews accompanying this article and as part of the series “Landscape review of maternal immunization”.

The reviews produced by the experts in the context of the landscape analysis were included in the final report to the BMGF. The publication of a series of articles in *The Lancet Infectious Diseases* broadened the dissemination of our results such as to reach medically trained professional worldwide. The series contains shorter versions of each domain expert’s review and included the major results of the survey for each domain.

Notably, the review process, from convening the expert reviewers to writing the final report, was completed within 6 months.

Discussion

To evaluate the needs of new or emerging areas of research, granting agencies periodically seek advice to determine the “state of the art”, identify knowledge gaps, and plan future directions. Advice-seeking takes many forms, including commissioned literature reviews, expert advisory panels and workshops as well as consensus-seeking meetings. Each approach has advantages and disadvantages. Literature reviews are a common starting point but can take considerable time to complete. Expert panels and workshops can produce useful guidance more quickly but

risks incompleteness and attendee biases. Consensus-seeking meetings may also be influenced by the expertise and personalities of the invited participants.

Evaluating the scientific foundation of maternal immunization posed unique challenges that we attempted overcome in innovative ways. Since the knowledge base is widely distributed among diverse specialties, we chose to engage 10 expert reviewers, each familiar with a particular aspect of this science. Dividing the literature review was to speed its completion, as would reliance on experts already familiar with their area. Using a scoping approach to select only literature relevant to the immunobiologic focus of our review also sped up the review process and synthesis of information. Reviewers were coached through these processes to maximize procedural uniformity. Most relished the opportunity to ensure mastery of their subject area and to learn from the other reviewers in the process.

The workshop meeting that we held was typical of expert workshops except each presenter had completed a formal review and synthesis of the assigned literature. Presentations were enriched by insights from separate in-depth discussions with regulators and manufacturers, who may have otherwise been more reluctant to speak at open meetings. The audience of invited experts discussed the presentations, adding their insights. This worked well: over 100 knowledge gaps were identified to be distributed across the spectrum of the science.

To be actionable, the list of gaps needed to be shortened and prioritized. We selected 45 for further consideration based on their direct relevance to vaccine development or program refinement. Our method of consensus-seeking on priorities was to invite the global community of maternal immunization-oriented researchers, policy-makers and manufacturers to rank the importance of each of these 45 gaps, using an online survey. Nearly 200 responded, representing about half of the identified world's experts on this topic. Such broad input reduced the risk of personal biases in the results. Importantly, rating scores were remarkably similar between self-reported experts and non-experts on specific items in the survey (e.g. maternal immunology) and between industry and other respondents. Twenty gaps were rated most important - a sufficiently small number to be considered for future studies. Given that future studies will be conducted around the globe, obtaining endorsement of research priorities by the global research community represents a significant strength of our review process, although we do not know if non-responders' views would have differed or if rankings would have differed had fewer gaps been included for consideration.

Lastly, it is noteworthy that the whole review process was completed in just less than 6 months, making it feasible to include all or portions of the method in future exercises to identify research priorities.

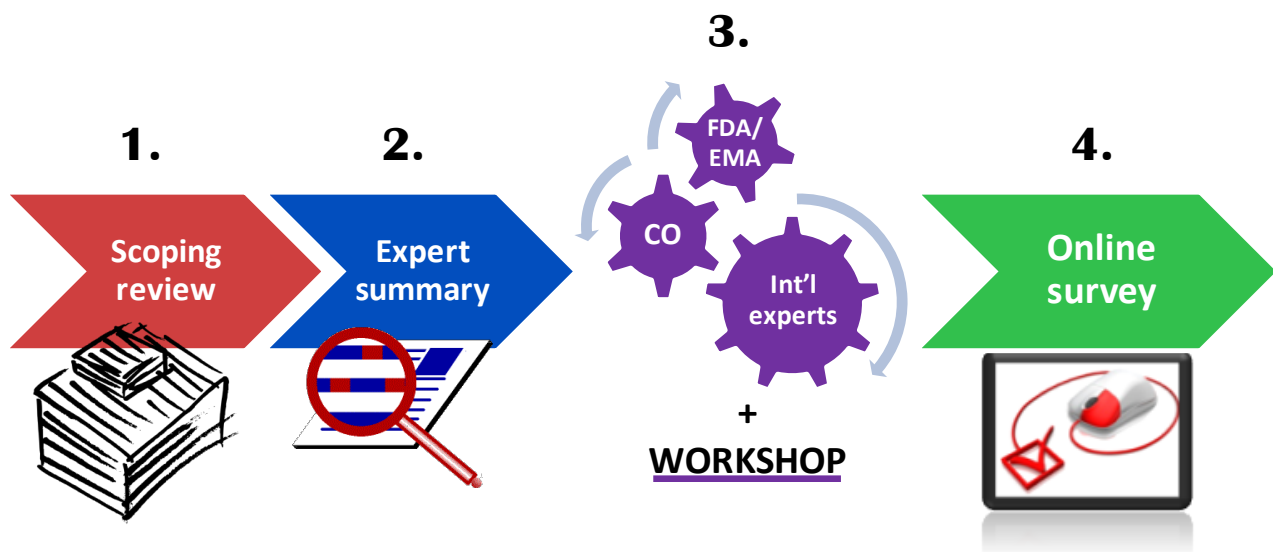
Conclusion

The unique approach developed here to rapidly conduct a landscape analysis was deemed successful based on *i)* the wide range of topics covered (immune response to vaccination during pregnancy; placental biology relevant to maternal immunization; maternal immunization and breastfeeding; fate and function of maternal antibodies in the fetus, newborn and infant; pertussis; GBS; RSV; influenza); *ii)* range of experts consulted (industry, regulators, academics, decisions makers, funders); and *iii)* consensus of the global community of experts in the field on a short list of actionable research priorities. The final report was provided to BMGF to help shape their future investments in maternal immunization research. Lastly, this effort also brought

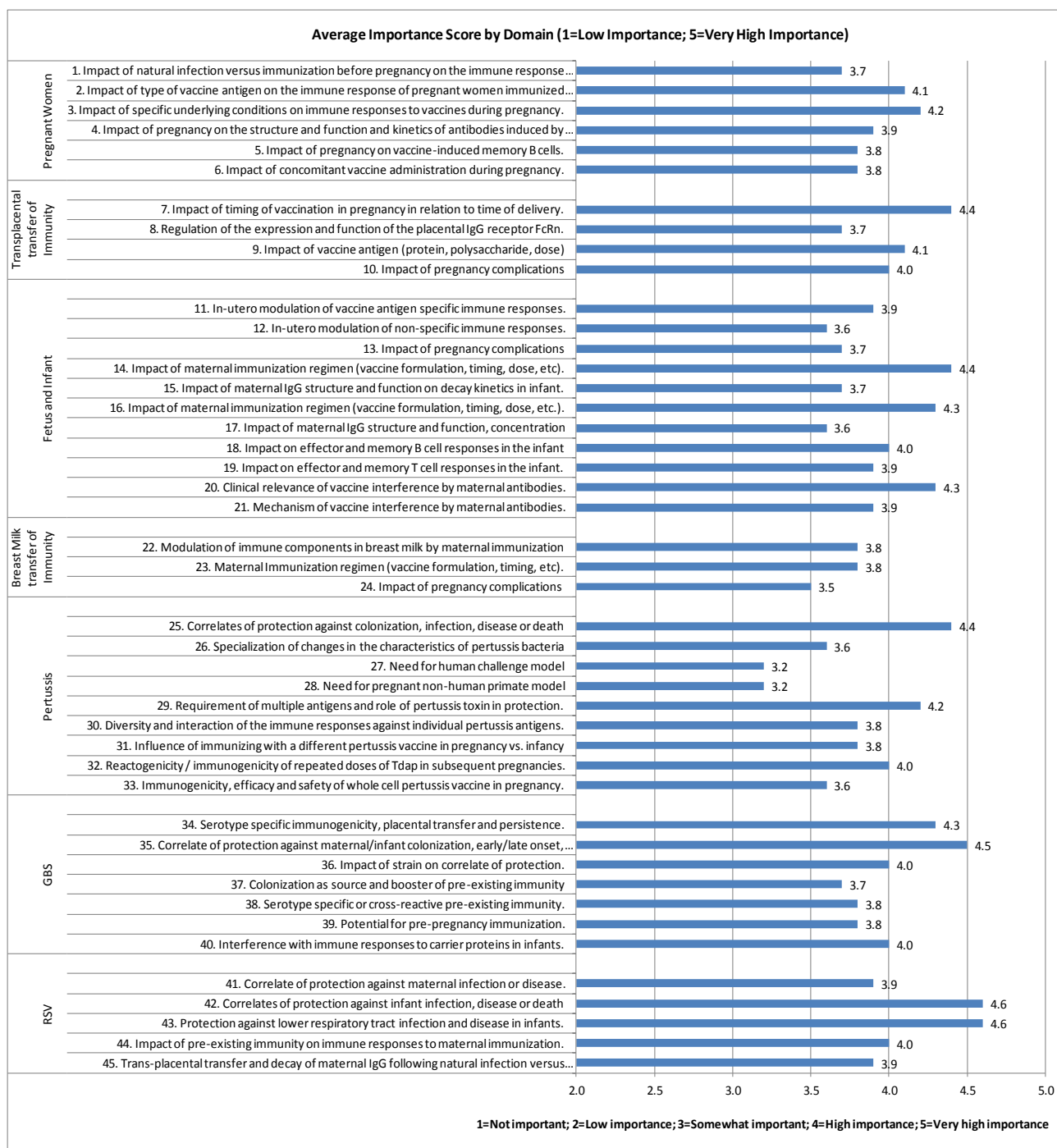
together, for the first time, experts across a wide range of disciplines relevant to maternal immunization. This unique amalgamation of individuals sharing a common interest and passion led to the natural and spontaneous formation of a global consortium of volunteers focused on advancing effective and safe maternal immunization. This consortium endorsed the landscape approach to maternal immunization and the unique processes used to produce the final report as described here.

Reference

1. Arksey H, O'Malley L. Scoping studies: towards a methodological framework. *Int J Social Research Methodology*. 2005; 8: 19-3



Supplemental Figure 1. Steps of the landscape review process. The first step was a scoping review of the literature to rapidly evaluate published data regarding maternal vaccination, summarized by each domain expert. To gain input from a wider range of stakeholders, maternal vaccine developers at 3 major vaccine companies (CO) and representatives from 2 key regulatory agencies (FDA, EMA) were consulted. Each domain expert's summary was presented to additional experts at a workshop, leading to identification of > 100 research gaps. Of these, 45 gaps considered most relevant for advancing vaccine development were included in an online survey. Nearly 200 global experts responded to the survey and ranked 20 gaps as most important for inclusion in future



Supplemental figure 2. Global Experts Survey response for importance of Knowledge Gaps identified.

Supplemental Table 1. Search strategy for literature review

Vaccinology and cross-talk	
#	Terms
1	cord blood immunoglobulins
2	passive immunity
3	immunoglobulins, passive
4	placental immunoglobulin transfer
5	neonatal immunoglobulins
6	immunity, mothers
7	Immunity, newborns
8	maternal antibodies
9	waning immunity
10	pregnancy, vaccination
11	vaccine
12	vaccination
13	immunisation
14	immunization
15	#11 or #12 or #13 or #14
16	pregnancy
17	maternal
18	#16 or #17
19	infant
20	neonate
21	newborn
22	#19 or #20 or #21
23	#15 and #18 and #22
Breast milk	
1	("mothers"[MeSH Terms] OR "mothers"[All Fields] OR "maternal"[All Fields]) AND ("immunisation"[All Fields] OR "vaccination"[MeSH Terms] OR "vaccination"[All Fields] OR "immunization"[All Fields] OR "immunization"[MeSH Terms]) AND ("milk, human"[MeSH Terms] OR ("milk"[All Fields] AND "human"[All Fields]) OR "human milk"[All Fields] OR ("breast"[All Fields] AND "milk"[All Fields]) OR "breast milk"[All Fields] OR "colostrum"[MeSH Terms]) AND English[lang]
Placenta	
1	exp mothers/ or maternal-fetal exchange/ or prenatal nutritional physiological phenomena/ or exp pregnancy, high-risk/ or exp pregnancy outcome/ or exp parturition/
2	(maternal or mother\$ or pregnancy).mp
3	#1 or #2
4	exp immunity/ or vaccination/ or immunomodulation/ or immunotherapy/ or exp immunization/

5	(immunization or immunisation) or vaccination or vaccine\$.mp
6	transfer adj3 (immunization or immunisation)
7	exp vaccines/
8	vaccine\$ or combined vaccine\$.mp
9	exp serology/
10	maternal-fetal exchange\$ or passive transfer or serology or antibody transfer.mp
11	(transfer adj3 (maternal or mother)).mp
12	#4 or #5 or #6 or #7 or #8 or #9 or #10 or #11
13	exp Infant, Newborn/
14	(neonate\$ or newborn\$).mp
15	#13 or #14
16	exp Placenta Accreta/ or exp Placenta, Retained/ or exp Placenta/ or exp Placenta Previa/ or exp Placenta Diseases/ or placentation/
17	placenta or placentation or afterbirth.mp
18	#16 or #17
19	immune system phenomena/ or antibody affinity/ or antibody diversity/ or antibody specificity/ or binding sites, antibody/ or exp dose-response relationship, immunologic/ or exp immune system processes/ or exp immunogenetic phenomena/ or exp lymphoid tissue/
20	antibod\$ isotype\$ or immunoglobulin\$.mp
21	exp antigens/ or exp microbiological processes/ or exp microbiota/ or exp maternal nutritional physiological phenomena/ or exp Breast Feeding/
22	pathogen or pathogens or prenatal nutrition or maternal nutrition.mp
23	#19 or #20 or #21 or #22
24	biological transport, active/ or facilitated diffusion/ or protein transport/ or secretory pathway/ or exp Receptors, Immunologic/ or endocytosis/ or exp transcytosis/ or exp Immunoglobulin Fragments/ or exp Absorption, Physiological/
25	(biological transport or Fc receptor\$ or endocytosis).mp
26	exp Infectious Disease Transmission, Vertical/ or exp risk factors/
27	mother to child transmission.mp
28	#24 or #25 or ##26 or #27
29	(((((#3 and #12) and #15) and #18) and #23) and #28)

Pregnancy

B cells & TFH biology in human or mice pregnancy and its potential impact on vaccine responses during pregnancy

1	"B-Lymphocytes"[Mesh]
2	"Pregnancy"[Mesh] and "Humans"[Mesh]
3	"Pregnancy"[Mesh] and "Mice"[Mesh]
4	1 AND 2
5	1 AND 3
6	"immunoglobulins"[mesh] AND "glycosylation"[mesh]
7	2 AND 6

8	3 AND 6
9	"Estrogens"[Mesh] OR "Progesterone"[mesh]
10	9 AND 1 AND 2
11	9 AND 1 AND 3
12	CD40 Ligand"[Mesh] OR "Inducible T-Cell Co-Stimulator Protein"[Mesh] "interleukin-21"[Supplementary Concept]
13	12 AND 2
14	12 AND 3
15	Immunoglobulin G"[Mesh] OR Immunoglobulin G/immunology"[Mesh] OR Immunoglobulin Isotypes"[Mesh]
16	"vaccination"[mesh] OR "immunization"[Mesh]
17	15 AND 16 AND 2 (limit to clinical trials)
18	15 AND 16 AND 3
Innate immunity, pregnancy and vaccines	
1	"Dendritic Cells"[Mesh]
2	"Monocytes"[Mesh]
3	"Immunity, innate"[Mesh]
4	Pregnancy"[Mesh]
5	"Humans"[Mesh] OR "Mice"[Mesh]
6	"Placenta"[Mesh]
7	(1 OR 2 OR 3) AND 4 AND 5 NOT 6
8	"Immunization"[Mesh]) OR "Vaccination"[Mesh]) OR "Vaccines"[Mesh] or "Adjuvant"[Mesh]
9	"interleukins"[Mesh]
10	(1 OR 2 OR 3 OR 9) AND 8 AND 4 AND 5
Clinical conditions in pregnancy & response to vaccines	
1	((("Pregnancy"[Mesh]) AND ("Vaccination"[Mesh] OR "Immunization"[Mesh] OR "Vaccines"[Mesh]) AND ("B-Lymphocytes"[Mesh] OR "Antibodies"[Mesh]) AND "Humans"[Mesh]))
2	Pre-eclampsia
3	Eclampsia
4	Diabetes
5	Obesity
6	Autoimmunity
7	Malnutrition
8	Asthma
9	Chronic hepatitis
10	Hepatitis B
11	Tuberculosis
12	Malaria
13	Hypergammaglobulinemia
14	Nutrition
15	1 AND 2
16	1 AND 3

17	1 AND 4
18	1 AND 5
19	1 AND 6
20	1 AND 7
21	1 AND 8
22	1 AND 9
23	1 AND 10
24	1 AND 11
25	1 AND 12
26	1 AND 13
27	1 AND 14
Neonates	
1	exp mothers/ or exp pregnancy/
2	(maternal or mother\$ or pregnancy).mp.
3	1 or 2
4	exp immunity/ or vaccination/ or exp immunization/ or exp vaccines/ or maternal-fetal exchange/
5	(immunization or immunisation or vaccination or vaccine\$ or combined vaccine\$ or maternal-fetal exchange\$ or passive transfer or antibody transfer).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]
6	(transfer adj3 (immunization or immunisation)).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]
7	4 or 5 or 6
8	exp Infant, Newborn/
9	(neonate\$ or newborn\$).mp.
10	8 or 9
11	exp antibody-producing cells/ or exp antigen-presenting cells/ or exp leukocytes/ or exp inflammation/ or exp cytokines/ or exp Immunoproteins/ or exp Inflammation Mediators/
12	(immune response or cytokine\$ or antibodies).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]
13	11 or 12
14	exp immune system phenomena/ or exp antigens/ or exp Absorption, Physiological/ or biological transport/ or exp Receptors, Immunologic/ or endocytosis/ or exp transcytosis/ or exp Immunoglobulin Fragments/

15	("antibody isotype" or immunoglobulin\$ or "antibody interference" or "antibody subclass" or "antibody half-life" or "antibody decay" or "antibody transport" or "Fc receptor").mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]
16	14 or 15
17	3 and 7
18	10 and 17
19	13 and 18
20	16 and 19
21	limit 20 to english language
22	limit 21 to yr="2010 -Current"

GBS

1	Maternal-Fetal Exchange/
2	Histocompatibility, Maternal-Fetal/
3	Maternal Serum Screening Tests/
4	Maternal Nutritional Physiological Phenomena/
5	immunity, maternally-acquired/
6	mothers/
7	(maternal or mother*).mp.
8	1 or 2 or 3 or 4 or 5 or 6 or 7
9	exp Vaccination/
10	immunization/
11	vaccination*.mp.
12	(immunization or immunisation).mp.
13	immunostimulation.mp.
14	(transfer adj3 (immunization or immunisation)).mp.
15	9 or 10 or 11 or 12 or 13 or 14
16	8 and 15
17	immunity, Innate/
18	innate immunity.mp.
19	Infant, Newborn/
20	(newborn\$ or infant\$).mp.
21	19 or 20
22	immune response.mp.
23	17 or 18 or 22
24	21 and 23
25	16 and 24
26	Group B streptococcus.mp.
27	Streptococcus agalactiae/
28	Streptococcal Infections/
29	26 or 27 or 28
30	25 and 29

31	16 and 21 and 29
Influenza	
1	exp mothers/ or maternal-fetal exchange/ or placentation/ or prenatal nutritional physiological phenomena/ or exp pregnancy, high-risk/ or exp pregnancy outcome/ or exp parturition/
2	(maternal or mother\$ or pregnancy).mp.
3	1 or 2 [Part1 Maternal]
4	exp immunity/ or vaccination/ or immunomodulation/ or immunotherapy/ or exp immunization/
5	(immunization or immunisation or vaccination or vaccine\$).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]
6	(transfer adj3 (immunization or immunisation)).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]
7	exp vaccines/
8	(vaccine\$ or combined vaccine\$).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]
9	exp serology/
10	(maternal-fetal exchange\$ or passive transfer or serology or antibody transfer).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]
11	(transfer adj3 (maternal or mother)).mp.
12	3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 [Part2 Immunization]
13	exp Infant, Newborn/
14	(neonate\$ or newborn\$).mp.
15	13 or 14 [Part3 Neonatal]
16	exp Influenza, Human/
17	(influenza or influenza B or influenza virus).mp.
18	16 or 17 [Part4 Influenza]
19	immune system phenomena/ or antibody affinity/ or antibody diversity/ or antibody specificity/ or binding sites, antibody/ or exp dose-response relationship, immunologic/ or exp immune system processes/ or exp immunogenetic phenomena/ or exp lymphoid tissue/ or exp Placenta/
20	(antibod\$ isotype\$ or immunoglobulin\$).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]
21	exp antigens/ or exp microbiological processes/ or exp microbiota/

22	(pathogen or pathogens).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]
23	19 or 20 or 21 or 22 [Part5 Immunobiological]
31	3 and 12 [Part 1+2]
32	31 and 15 [Part (1+2)+3]
33	32 and 18 [Part (1+2+3)+4]
34	33 and 23 [Part (1+2+3+4)+5]
35	limit 34 to (english language and yr="2000 -Current")
Pertussis	
1	mothers[Mesh] or Pregnancy[Mesh] or mothers[All Fields] or maternal[All Fields] or pregnancy[All Fields]
2	Immune System Phenomena[Mesh] or "vaccination"[All Fields] or "Vaccines"[Mesh] or "combined vaccine"[All Fields] or "Serology"[Mesh] or "maternal-fetal exchange"[All Fields] or "passive transfer"[All Fields] or "serology"[All Fields] or "antibody transfer"[All Fields] or "Receptors, Immunologic"[Mesh] or "models, animal"[MeSH Terms] or "immune system phenomena"[MeSH Terms] or "immune system phenomena"[All Fields] or "antibody affinity"[MeSH Terms] or "antibody affinity"[All Fields] or "antibody diversity"[MeSH Terms] or "antibody diversity"[All Fields] or "binding sites, antibody"[MeSH Terms] or "antibody binding sites"[All Fields] or "immune system processes"[MeSH Terms] or "immune system processes"[All Fields] or "immunogenetic phenomena"[MeSH Terms] or "immunogenetic phenomena"[All Fields] or "lymphoid tissue"[MeSH Terms] or "lymphoid tissue"[All Fields] or "antigens"[MeSH Terms] or "antigens"[All Fields] or "microbiological processes"[MeSH Terms] or "microbiological processes"[All Fields] or "immunoglobulins"[All Fields] or "immunoglobulin"[All Fields]
3	#1 and #2
4	"Infant, Newborn"[Mesh] or "neonate"[All Fields] or "neonates"[All Fields]
5	#3 and #4
6	or "Infant, Newborn"[Mesh] or "neonate"[All F "Whooping Cough"[Mesh] or "Virulence Factors, Bordetella"[Mesh] or "Defensins"[Mesh] or "Host-Pathogen Interactions"[Mesh] or "Fimbriae, Bacterial"[Mesh] or "Fimbriae Proteins"[Mesh] or "whooping cough"[All Fields] or "Pertussis toxin"[All Fields] or "defensins"[All Fields] or "Host-Pathogen Interactions"[All Fields] or "Filamentous hemagglutinin"[All Fields] or "pertactin"[All Fields] or "fimbriae"[All Fields] ields] or "neonates"[All Fields]
7	#5 and #6
RSV	
1	Search maternal or mother* or pregnancy
2	Search "parturition"[mesh]
3	Search "prenatal nutritional physiological phenomena"[mesh]
4	Search "placentation"[mesh]

5	Search "mothers"[mesh]
6	Search "pregnancy"[mesh]
7	Search "maternal-fetal exchange/immunology"[mesh]
8	Search "maternal-fetal exchange"[mesh]
9	#1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8
10	Search (antibod* OR immunoglobulin*)
11	Search immune response
12	Search "immunity, mucosal"[mesh]
13	Search "immunity, innate"[mesh]
14	Search "neutralization tests"[mesh]
15	Search "lung/ immunology"[mesh]
16	Search "immunity, maternally acquired"[mesh]
17	Search "immunity, cellular"[mesh]
18	Search "immunity, active"[mesh]
19	Search "Cytokines"[mesh]
20	Search "CD8 positive t lymphocytes"[mesh]
21	Search "CD4 positive t lymphocytes"[mesh]
22	Search "antibody specificity"[mesh]
23	Search "immunization, passive"[mesh]
24	Search "immunoglobulins"[mesh]
25	Search "antibodies"[mesh]
26	#10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19 OR #20 OR #21 OR #22 OR #23 OR #24 OR #25
27	Search RSV
28	Search respiratory syncytial virus
29	Search "respiratory syncytial virus infections"[mesh]
30	Search "respiratory syncytial virus vaccines"[mesh]
31	Search "respiratory syncytial virus, human"[mesh]
32	Search "respiratory syncytial viruses"[mesh]
33	#27 OR #28 OR #29 OR #30 OR #31 OR #32
34	#9 AND #26 AND #33

Supplemental Table 2. Results from the literature search and selection process (Number of articles)

Steps (in processing order)	Vaccinology and cross-talk	Breast milk	Placenta	Pregnancy	Neonates	GBS	Influenza	Pertussis	RSV
Initial search	2859	311	108	3808	547	110	54	189	282
General exclusion criteria: language, publication date, etc	349	162	78	248	547	30	54	105	129
Full text reviewed after abstract screening	211	68	78	119	179	30	54	49	57
Additional references added from citations, personal collection, as advised by other members of consortium*		20		28	26	108		33	27
Articles used for final report	53	88	28	86	115	138	32	48	84

*Blank space denotes information not available.

Supplemental Table 3. Conceptual categories created during the review process by each topic area (reported when applicable)

Vaccinology and cross-talk

	Categories
1	Response to immunization in pregnancy
2	Placental transfer of antibodies
3	Neonatal issues relating to maternal immunization
4	Infant protection
5	Infant immunization in the context of maternal immunization/maternal antibodies
6	Enhancing vaccine programme development
7	Public involvement

Pertussis

	Categories	Sub-theme
1	Protective Antigens:	
		PT
		FHA
		PRN
		Agglutinogens
2	Mechanism of immunity: antibody response placental transfer kinetics:	
		PT
		FHA
		PRN
		FIM
		ACT
		Agglutinogens
3	Mechanism of immunity: Impact of timing of vaccination during pregnancy:	
		antibody half life post-partum
		prematurity vs. term
		antibody levels during pregnancy
4	Mechanism of immunity: Effect on active immunization of infant:	
		presence or absence of interference
5	Breast milk:	
		transfer of antibody to breast milk
		activity of breast milk against B. pertussis
6	Whole cell vs. acellular pertussis vaccine:	

	whole cell vs acellular
--	-------------------------

RSV

	Categories
1	Does maternal antibody protect infants against RSV infection and, if so, for how long?
2	Is infant protection due to maternal antibody, or might other factors explain the association?
3	What are the relative contributions of breast milk and transplacental antibody transfer?
4	What is the most relevant and appropriate antibody to measure, and how?
5	What do animal models tell us?
6	Could maternal antibody interfere with infant immune responses to RSV vaccines or infection?
7	What gaps in knowledge are there?

-

Supplemental Table 4. Demographic characteristics of the survey respondents.

Primary Affiliation	Researcher N=123	Decision maker N=10	Research Support N=8	Other expert N=53	Total N=194
University	97	3	4	18	122
Government	13	4	2	16	35
Industry	4	2	1	6	13
NGO	8	1	1	10	20
Other & None provided	1	0	0	3	4
Consider themselves an expert (multiple selections are allowed)					
Influenza	62	5	2	29	98
Pertussis	52	7	1	30	90
RSV	53	4	0	23	80
GBS	25	1	3	12	41
Placental biology	6	0	1	1	8
Breast milk biology	9	1	0	3	13
Maternal immunology	26	0	1	11	32
Neonatal immunology	35	3	1	14	53
Primary Specialization					
Immunologist	32	2	3	5	42
Clinician	29	5	2	15	51
Clinical Trial	26	0	0	9	35
Microbiologist	7	1	1	5	15
Epidemiologist	23	2	2	15	42
Social Scientist	3	0	0	0	3
Program Manager /Administrator	2	0	0	2	4
Other	1	0	0	1	2

In the last 2 years, were you involved in	Yes (%)	No (%)	Total (%)
Maternal Immunization Research?	129 (66%)	65 (34%)	194 (100%)
Basic Science/immunology based?	52 (40%)	77 (60%)	129 (100%)
Clinical Trials?	70 (54%)	59 (46%)	129 (100%)
Programmatic Study/evaluation?	48 (37%)	81 (63%)	129 (100%)
Social Science?	15 (12%)	114 (88%)	129 (100%)
Policy?	24 (19%)	105 (81%)	129 (100%)
Other?	1 (1%)	128 (99%)	129 (100%)

Appendix 1. 108 knowledge gaps identified during search, presentations, and discussion sessions.

Overall highlights of the gaps:

1. Need clinical disease definitions for future studies
2. Global disease burden
3. Current situation of advisory groups leapfrogged regulators:
 - e.g. current Tdap recommendations expedient but with possible handicap for controlled studies in pregnant women
4. Ecological evidence:
 - e.g. opportunity to use actual experience as part of credible evidence
5. Advocacy group for maternal immunization:
 - e.g. mediator/connector between industry, regulators, funders, academics public health
6. We need qualified (standardized) assays

Pregnant women

1. Most vaccines will target pathogens against which pregnant women have pre-existing immunity. Rate the importance and feasibility of filling the following knowledge gaps:
 - Enhancement or suppression of vaccine responses by pre-existing immunity
 - Impact of natural infection versus vaccination before pregnancy on the quality and boost-ability of pre-existing immunity
 - Impact of type of antigen (protein, polysaccharide) on the quality and boost-ability of pre-existing immunity
2. Pregnant women can be a reservoir of pathogens. Rate the importance and feasibility of filling the following knowledge gaps:
 - Impact of pregnancy on pathogen reservoir
 - Impact of vaccination before or during pregnancy on pathogen reservoir
3. Maternal characteristics, health and infections during pregnancy may impact immune responses to vaccines. Rate the importance and feasibility of filling the following knowledge gaps:
 - Impact of the global burden of infectious pathogens in low, middle and high income countries on immune responses to vaccines during pregnancy
 - Impact of specific pathogens (HIV, malaria, chronic hepatitis, helminthiasis,...) on immune responses to vaccines during pregnancy
 - Impact of immune dysregulation (hypergammaglobulinemia, immune cell exhaustion, autoimmunity,...) on immune responses to vaccines during pregnancy
 - Impact of age or parity on immune responses to vaccines during pregnancy
 - Impact of nutrition on immune responses to vaccines during pregnancy
4. Pregnancy may impact immune responses to vaccines. Rate the importance and feasibility of filling the following knowledge gaps:
 - Impact of pregnancy on the structure and function of antibodies induced by vaccines
 - Impact of pregnancy on the decay of antibodies induced by vaccines
 - Impact of pregnancy on vaccine-induced memory B cells
 - Impact of pregnancy on innate immune responses and inflammation induced by vaccines

- Impact of timing of vaccination during pregnancy on immune responses to vaccines
5. The induction of protective immune responses during pregnancy may require the use of adjuvants. Rate the importance and feasibility of filling the following knowledge gaps:
- Impact of adjuvants on adaptive immune responses to vaccines during pregnancy
 - Impact of adjuvants on innate/inflammatory responses during pregnancy

Transplacental transfer of immunity

1. Several factors may influence the trans-placental transfer of IgG. Rate the importance and feasibility of filling the following knowledge gaps regarding the trans-placental transfer of IgG:
 - Impact of timing of vaccination during pregnancy
 - Impact of vaccine antigen (protein, polysaccharide)
 - Impact of vaccine antigen dose
 - Impact of antigen priming (vaccine or pathogen) before pregnancy
 - Impact of adjuvants
 - Impact of maternal IgG structure
 - Impact of hypergammaglobulinemia
 - Impact of pathogens other than HIV or malaria
 - Impact of pregnancy complications (preeclampsia, preterm labor, infections, premature delivery..)
2. Maternal IgG are transported by the neonatal Fc receptor (FcRn) through the placenta. Rate the importance and feasibility of filling the following knowledge gaps:
 - Regulation of the expression of the FcRn by syncytiotrophoblasts
 - Factors impairing the development of the placenta
 - Impact of pathogens on the development and function of the placenta
 - Impact of the placental microbiome on its development and function
 - Transport of antigen-IgG complexes through the placenta

Fetus and infant

1. Maternal immunization may impact the fetal immune system. Rate the importance and feasibility of filling the following knowledge gaps:
 - In-utero priming or suppression of vaccine antigen specific immune responses
 - In-utero priming or suppression of non-specific immune responses
2. Maternal antibodies provide protective immunity in the infant. Rate the importance and feasibility of filling the following knowledge gaps:
 - Distribution of maternal antibodies in the infant (systemic, mucosa,..)
 - Role of maternal antibodies in the defence against respiratory pathogens
 - Potential induction of active immunity in the infant by attenuation of natural infection (passive-active immunization)
3. Several factors may influence the decay of maternal IgG in infants. Rate the importance and feasibility of filling the following knowledge gaps regarding IgG decay:

- Impact of environment (low versus middle or high income countries)
 - Impact of maternal infections (HIV, malaria,..)
 - Impact of prematurity
 - Impact of timing of immunization in the mother
 - Impact of antigen used to immunize the mother (protein, polysaccharide)
 - Impact of adjuvants used to immunize the mother
 - Impact of antigen priming (vaccine or pathogen) before pregnancy
 - Impact of IgG structure and function
4. Maternal antibodies may interfere with vaccine responses in infants. Rate the importance and feasibility of filling the following knowledge gaps regarding vaccine interference:
- Impact of antigen used to immunize the mother (protein, polysaccharide)
 - Impact of adjuvants used to immunize the mother
 - Impact of antigen priming (vaccine or pathogen) before pregnancy
 - Impact of maternal IgG concentration
 - Impact of maternal IgG structure and function
 - Impact on effector and memory B cell responses
 - Impact on effector and memory T cell responses
 - Clinical relevance of vaccine interference by maternal antibodies
 - Mechanism of vaccine interference by maternal antibodies
 - Animal models of vaccine interference by maternal antibodies

Breast milk transfer of immunity

1. Breastfeeding provides protective immunity in the infant. Rate the importance and feasibility of filling the following knowledge gaps:
- Impact of breastfeeding on immunity at the systemic versus mucosal levels
 - Potential induction of active immunity in the infant by attenuation of natural infection (passive-active immunization)
 - Components of breast milk providing protection in infants
 - Regulation of immune components in breast milk
 - Measurement of breast milk components in vaccine trials
2. Several factors may influence the transfer of immunity through breast milk. Rate the importance and feasibility of filling the following knowledge gaps regarding breast milk transfer of immunity:
- Impact of maternal immunization
 - Impact of timing of immunization in the mother
 - Impact of antigen used to immunize the mother (protein, polysaccharide)
 - Impact of adjuvants used to immunize the mother
 - Impact of antigen priming (vaccine or pathogen) before pregnancy
 - Impact of environment (low versus middle or high income countries)
 - Impact of maternal infections (HIV, malaria,..)
 - Impact of prematurity

Pertussis

1. Correlates of protection would help the implementation of maternal immunization against pertussis and the evaluation of vaccine candidates. Rate the importance and feasibility of filling the following knowledge gaps regarding correlates of protection:

- Correlate of protection against colonization, infection, disease or death
 - Role of T lymphocytes in protective immunity against pertussis
 - Role of soluble factors (cytokines,...) in serum or breast milk
 - Role of changes in the characteristics of pertussis
 - Need for human challenge model
 - Need for pregnant non-human primate model
2. Different pertussis vaccine components and vaccines may have different immunogenicity and efficacy in pregnant women. Rate the importance and feasibility of filling the following knowledge gaps:
- Requirement of multiple antigens and role of pertussis toxin in protection
 - Diversity of the immune responses against individual pertussis antigens
 - Interactions between immune responses to individual pertussis vaccine antigens
 - Influence of immunizing with a different pertussis vaccine in pregnancy and in infancy
 - “Reactogenicity” of repeated doses of Tdap in subsequent pregnancies
 - Immunogenicity, effectiveness and safety of whole cell pertussis vaccine in pregnancy

Influenza

1. Correlates of protection may help the implementation of maternal immunization against influenza. Rate the importance and feasibility of filling the following knowledge gaps:
- Correlate of protection against maternal infection or disease
 - Correlate of protection against infant infection, disease or death
 - Impact of maternal HIV infection on correlate of protection against infection or disease
2. Influenza infection during pregnancy may have several impacts on the infants. Rate the importance and feasibility of filling the following knowledge gaps:
- Mother to infant transmission of influenza
 - Prevention of adverse fetal outcomes of maternal influenza infection (low birth weight, prematurity,...) by maternal immunization
 - Correlate of protection against adverse fetal outcomes induced by maternal influenza infection
 - Impact of maternal infection on infant susceptibility to disease
3. Several factors may impact the immunogenicity of influenza vaccines during pregnancy. Rate the importance and feasibility of filling the following knowledge gaps:
- Immunogenicity of different influenza virus strains
 - Impact of concomitant influenza and pertussis vaccination
 - Primary immune responses to pandemic influenza vaccines during pregnancy

Group B streptococcus

1. Correlates of protection would help the evaluation of candidates for maternal immunization against GBS. Rate the importance and feasibility of filling the following knowledge gaps regarding correlates of protection:
- Correlate of protection against maternal colonization
 - Correlate of protection against infant early or late onset infection, disease or death

- Correlate of protection in breast milk
 - Impact of maternal immunization on other infant outcomes than sepsis
 - Impact of strain virulence on correlate of protection
 - Optimal assay to define correlate of protection
 - Role of maternal IgG isotype
2. Pre-existing immunity may impact the immunogenicity of maternal immunization against GBS. Rate the importance and feasibility of filling the following knowledge gaps:
 - Impact of carriage on pre-existing immunity
 - Serotype specific or cross-reactive pre-existing immunity
 - Potential for pre-pregnancy immunization
 - Impact of pre-existing immunity against carrier proteins
 3. Conjugate vaccines are potential candidates for maternal immunization against GBS. Rate the importance and feasibility of filling the following knowledge gaps:
 - Serotype specific immunogenicity
 - Immunogenicity of two dose schedules
 - Interference with immune responses to carrier proteins in infants

Respiratory syncytial virus

1. Correlates of protection would help the evaluation of candidates for maternal immunization against RSV. Rate the importance and feasibility of filling the following knowledge gaps:
 - Correlate of protection against maternal infection or disease
 - Correlate of protection against infant infection, disease or death
 - Protection against lower respiratory tract infection and disease in infants
2. Infection with RSV is universal and induces incomplete immunity. Rate the importance and feasibility of filling the following knowledge gaps:
 - Impact of pre-existing immunity on immune responses to maternal immunization
 - Trans-placental transfer and decay of maternal IgG following natural infection versus maternal immunization

Point by point reply to the reviewers of the three combined manuscripts

Manuscript 1. Maternal immunization strategies: Improving on Mother Nature (THELANCETID-D-00253)

Reviewer #1:

- Overall this is a well written review on maternal immunization highlighting the gaps and areas for further research. Title sounds perhaps to positive and could end with a question mark, as a lot still needs to be further explored.

The title of the combined manuscript takes into account the helpful suggestion of the Reviewer.

- Abstract: mentions 'immunizing women 'before'pregnancy: but as far as I understand this is not exactly an convincing added value. So I would delete it from the abstract.

This part has been deleted from the abstract of the combined manuscript.

- introduction, p3: line 18-21: put it in chronological sequence, as the most convincing part of evidence comes from the tetanus vaccination in pregnant women, followed by influenza, then pandemic influenza and pertussis.

This section has been re-ordered as suggested by the Reviewer and has been shortened in the combined manuscript (p4, line 12) to meet the recommendation of the Editor on the total wordcount.

- what I miss here is the recent discussion at WHO and SAGE level on the influenza immunization in pregnant women, where recommendations have been made softer, or at least adapted to the regions. This should be reflected here too.

The suggestion of the Reviewer has not been incorporated in the combined manuscript because of the recommendations of the Editor to limit overlap with the two disease-specific reviews and to limit the total wordcount of the manuscript.

- page 4, line 7: effectiveness in whom and how defined?

The point about « in whom » is clarified (p21, line 3).

page 5, line 7: what I miss here at the end of the introduction is a paragraph on the clear need to document the burden of neonatal and infant morbidity and mortality globally and in particular in LMIC to justify maternal immunization policies against pertussis and influenza for instance.

The suggestion of the Reviewer could not be incorporated in the combined manuscript because of the recommendation of the Editor to limit the total wordcount. We indicate in the combined manuscript that a discussion of the burden of disease is beyond the scope of the manuscript (p6, line 21).

page 6, line 8: please specify other backgrounds

This point is clarified (p6, line 10).

page 6, line 20: sufficient and timely

This text has been added (p7, line 1).

page 7, line 11: the authors say: where disease burden is highest, but this needs still to be proven in neonates and infants for some vaccine-preventable diseases, or at least the added value of maternal immunization in preventing such burden versus the current infant immunization programmes needs to be proven (see comment above)

References to disease burden have been removed from the combined manuscript. See reply to comment above.

page 8, last sentence: is affinity and avidity something that the average reader will understand - short explanation could help perhaps.

This is clarified (p22, line 7).

page 9, line 3, ref 14: please also look at the letter to the editor reacting on this paper and incorporate that comment in your text or gap analysis.

A reference to the letter is included (p11, line 14).

page 9: forelast sentence: there is enough evidence that shows that repeated boosters or dTpa injections do not increase severe injection site reactions. So, please adapt according to the recent literature, as ref 16 refers to a paper from 1979.

The sentences have been removed from the combined manuscript.

p10, is not clear to me: is there a monovalent aP vaccine licensed in Europe? Please make that more explicit.

The sentence has been removed from the combined manuscript.

p10, line 15: relative contra-indication is true for attenuated vaccines, not for inactivated vaccines as hepB, hepA, tetanus vaccines were already given to pregnant women when indicated.

The sentence has been removed from the combined manuscript.

p13: the part on placental transfer should be completed by a part on breastfeeding/milk transfer and explain how this articulate with the placental transfer

A section on « Transfer of maternal immunity through breastfeeding » is included in the combined manuscript.

p16, line 9: 'protective' is mentioned (also on line 12): but for pertussis no correlate of protection is defined or known, so please rephrase;

The sentences have been removed from the combined manuscript.

Reviewer #2:

This invited review presents a meeting report on Maternal vaccination that was held in Vancouver. This overview report, appears to be a prelude to a series of diseases and topic specific articles planned for Lancet Infectious Disease.

The manuscript is generally well written.

Minor comments:

1. Under summary:- suggest adding RSV to the 2nd line

This has been added.

2. Pg 3- its important not to overplay the role of maternal tetanus vaccination as a measure of potential for other maternal vaccines. In particular, although maternal TT vaccination has contributed to the decline in neonatal tetanus, the magnitude of the decline far exceeds that compared to the change in coverage with tetanus vaccination in low income settings. The overall decline is likely due to a combination of maternal TT vaccination, coupled with changes in birth practices and other cultural factors which had previously predisposed to neonatal tetanus. This should be clarified- Pg 3 , 3rd last line.

This section has been shortened in the combined manuscript (p4, line 12) to meet the recommendation of the Editor on the total wordcount.

3. Pg 8 2nd last para: The timing of maternal vaccination remains controversial. Recent published data on influenza (Nunes M et al - JID 2015) and presented data on Novovax RSV vaccine, indicate that there is a higher concentration of antibody transferred the longer the time between vaccination and delivery. So although the antibody peak might be higher at time of delivery, the closer the vaccination is given in relation to delivery, the efficiency of transfer and net concentration accumulated in the newborn could be higher the earlier that vaccination is give during pregnancy (at least moving into the 2nd trimester).

This point is now discussed, with particular reference to pertussis immunization (p12, line 1)

4. Figure- The pre-immunization data point on the Figure, although understandably only illustrative, probably should be reconsidered to being shown to be below the CoP threshold, otherwise there is very little reason to be immunizing.

The figure has been modified following the helpful suggestion of the Reviewer. This figure is Figure 2 in the combined manuscript.

Reviewer #3:

The manuscript is the introductory part of a series on different aspects of immunization in pregnancy. As a reader, I expect that the other articles will develop specific topics more in deep.

In fact, it is not easy to critically review the findings in this paper since many aspects of immunization in pregnancy are not fully developed.

The title is appealing, though "improving on Mother Nature" may seem misleading. We are still far from doing better than the natural complex mechanism that protects newborns from infectious diseases.

The title of the combined manuscript takes into account the helpful suggestion of the Reviewer.

Beside this comment, I have some suggestions for Authors that may improve the readability of the article:

- Antibody transfer from mothers is obviously extremely important. What we don't know yet is which role other factors may play, including cell transfer and other unspecific mechanisms of protection pertinent to innate immunity. Spending some words in the Introduction on the very complex mechanism underlying newborn protection and on the interaction mother-newborn may be appropriate;

This point is discussed in the section on « Maternal immunization and infant immunity ».

- The process for identifying research gaps is anticipated in this introductory article although its methodological steps are described in detail in another article of the same series. Although the description of this process seems sound, there is much emphasis on the personal role of the reviewers and of the expert group on the prioritization of knowledge gaps. Though the scope of my review is not commenting on other papers in the same issue of Lancet Infectious Diseases, I wonder if there has been any additional validation process in the identification of research gaps;

As indicated in the combined manuscript (p6, lines 7-14 and Supplemental materials), the landscape analysis included an online survey completed by nearly 200 « content experts » who prioritized research gaps identified at the collaborative workshop.

- Considering the priorities indicated in Table 1, I am surprised that some obvious questions are not included. For example, the issue of safety of immunizations in pregnancy (although much reconsidered) is just touched for repeated pertussis vaccines. Another important point would be perception of immunization by the target group and potential immunization strategies, since it is not obvious how much vaccination in pregnancy will be acceptable in different settings and how it would be integrated in existing programmes. Another point that still deserves attention is what are the determinants of lack of response to immunization in pregnancy;

We agree with the Reviewer that these are all important points. As indicated in the Introduction of the combined manuscript, the landscape analysis focused on the immunobiology of maternal immunization and other crucially important aspects of maternal immunization—safety, public perception, and integration into existing global immunization programs—were outside the scope of the project. On the other hand, immune responses to vaccines in pregnancy are discussed in the section “Impact of pregnancy on vaccine responses”.

- It is quite obvious that immunization in pregnancy against different pathogens are diverse (Table 2) given the different pathophysiology of different infectious agents. The main consequence of this observation would be that combination vaccines to be administered in pregnancy would be difficult to develop. Although we are far away from this concept, it may be

worthwhile to suggest the practical implications of these observations in the paragraph "Maternal immunization strategies";

The suggestion of the Reviewer could not be incorporated in the combined manuscript because of the recommendation of the Editor to limit the total wordcount.

- In the paragraph on "Immunogenicity and safety" the Authors underline how a single shot would be sufficient in most cases to elicit a sufficient (protective?) response. Indeed, we are not yet completely confident that this is the case. In particular much remains to understand about the role of B memory cells in some diseases. A comment on this point would be useful to readers;

This part has been deleted from the combined manuscript to follow the recommendation of the Editor to limit the total wordcount.

- The Authors also elaborate in the same paragraph on the balance between high Ab concentration and avidity. The paper to support this observation (Ref 14) used a method to measure avidity that is a modified ELISA and not a direct measure of binding avidity/affinity. Other Authors had different results (Maertens Vaccine 2015, 33:5489). In addition and most importantly there is no data on the correlation between protection and avidity. On the contrary, it has been shown that in vivo antibody concentration is the most important factor of protection (Bachmann MF, Science 1997, 276:2024-2027). I suggest that this section is expanded to consider the controversies on this topic;

A reference to the letter by Maertens is included in the manuscript (p11, line 14). We did not further discuss aspects related to pertussis in order to follow the recommendation of the Editor to limit overlap with the two disease-specific reviews. Some discussion about correlates of protection is included in the section on «Maternal immunization and infant immunity».

- Important considerations must be also made for the kinetics of Ab levels. The Authors may comment on the benefits (and the resulting strategies) of being immunized while planning a pregnancy vs during pregnancy. We should not forget also that some pathogens have potential harmful effects on the fetus (influenza and spontaneous abortion or preterm delivery);

The point of kinetics of vaccine responses during pregnancy is now discussed in the section on « Impact of pregnancy on vaccine responses ». A comment is also included on pre-pregnancy immunization (p7, line 10).

- In the paragraph "Placental transfer of antibodies", the Authors suggest that Ab with high avidity could be preferentially transferred through the placenta. The references in support of this observation are however quite old. It is difficult to imagine how the Fc receptors transporting IgG are monitored by the placenta and taken into account for affinity/avidity against any antigen. Moreover, IgG1 is the major isotype in mothers and children and most immunization results in the production of IgG1 while natural infections generate other isotypes. A comment on this would be useful for the readers;

These points are discussed and a more balanced view is provided in the section on « Transfer of maternal immunity through the placenta ».

- In the paragraph "Impact of maternal immunization" the data on the MF59-adjuvanted H1N1 influenza vaccine show a reduction of inflammatory cytokines in the nasal fluid of the children.

This, rather than impaired immunity, may be due to a reduced exposure to infections (vaccinated and maybe more careful mothers) and thus just show reduced local inflammation. No effect on subsequent child immune responses was shown. This should be considered in discussing the findings of the study;

This point has been removed from the combined manuscript.

- I agree that breast feeding is one of the components affecting protection of the infant (at least for some diseases). Still, we do not have enough information to understand the complex interplay with immunization in pregnancy. Since breast feeding may be extremely important for developing countries, its role deserves priority in future studies;

A section on « Transfer of maternal immunity through breastfeeding » is included in the combined manuscript.

- I do not agree that knowledge gaps should be addressed through clinical trials only. Although this is highly desirable, it would be extremely difficult to design experimental studies focused on these topics. Observational well designed studies are still a significant source of information that should not be disregarded.

This point has been removed from the combined manuscript.

Reviewer #4:

1) It is not clear to the reader if this article is intended to be an introduction (editorial) to an article series on maternal immunisation, or a "stand-alone" article in the series. It starts out as an introduction to the topic of maternal immunisation and this impression is strengthened when the titles of the other 7 articles are listed on page 4, but the rest of the article doesn't evolve in that direction. I suggest you try to make it clear what the article is by developing it further in one or the other direction.

This point is clarified in the combined manuscript (p5, lines 4-11).

There is an excessive use of adjectives throughout the article, most of them unjustified. It gives the impression that the authors are trying to inflate the importance of what is being stated and there is really no need for that.

What is a "scoping review" and why is it within citation marks?

The combined manuscript takes into account the comment of the Reviewer and the definition of a scoping review is provided in the Supplemental materials.

After reading the list with the other articles in the series, I can't help asking what justifies this article. The titles will deliver what they indicate regarding the concerned diseases (pertussis, influenza, RSV and GBS), then I can't see the reason for repeating it in this article.

Following the suggestion of the Editor, the manuscript has been combined with two other manuscripts describing the methodology of the landscape analysis, the identified research gaps and the results of the landscape analysis on the immunobiology of maternal immunization.

I suggest you structure the article as a review of the current knowledge of transplacental transfer of maternal antibodies and transplacental transfer of vaccine antigens. Avoid jumping from one antigen to the next. Start with the basic concepts and drill down to the specific details and the gaps.

The suggestion of the Reviewer has been taken into account in the preparation of the combined manuscript.

Manuscript 2. An innovative approach to determine research priorities in maternal immunization through international collaboration (THELANCETID-D-16-00249)

Reviewer #1:

It was a pleasure reading through this paper and it is really impressive of coming up with such imperative gaps from the work of six months only. However, the link to the actual scoping reviews generated from the exercise would have helped as well. Which at the moment was not provided in the paper.

Following the recommendation of the Editor, scoping reviews are now described in three individual manuscripts. Reference is made in the text of this manuscript to the other two companion manuscripts (p5, line 6 and Supplemental materials)

Few of the minor comments might help in improving the presentation of the paper.

Table 1:

It is important if it is described somewhere in the text or in the table that why it was elected to include papers and literature published from the year 2000 onwards.

At one place in Table 1, it is marked in asterisk since 1996 and since 1985? For what exact cells those signs are?

This point has been corrected and Table 1 has been corrected as Panel 1 in Supplemental materials, as recommended by the Editor.

Also as a foot note it is mentioned that non English articles were not excluded, however under the exclusion criteria it is mentioned that Papers/articles in Non English language were excluded and it was one of the exclusion criteria. There is some discrepancy.

This point has now corrected.

Is it also possible that from among those 108 research gaps that were identified, to show some questions which had 0 or lowest scores?

So if those had lowest scores, then it is important to understand why those were identified as gaps at the first place?

Among the 108 gaps identified through the literature review and collaborative workshop, 45 were considered as most relevant during the collaborative workshop and were included in the online survey. The 63 gaps that were not included in the survey were therefore not scored. The basis for the selection of the 45 gaps is explained in the combined manuscript (p6, line 7 and Supplemental materials).

A minor point to correct: remove underscore from maternal_immunization on Page 4.

This has been corrected.

Reviewer #2:

This paper provides a description of a review process undertaken by researchers to identify gaps in knowledge related to the immunobiology of maternal immunisation. Interesting paper, written as a protocol for identifying research gaps, using a landscape review and consultative process, in this case describing research gaps in maternal immunisation.

The paper is similar in content and outcomes to a previous paper "maternal immunisation-opportunities for scientific advancement" published in CID and authored by one of the authors of this manuscript which details the outcome of a series of meetings to discuss priorities for research in maternal immunisation. The findings of both papers are not dissimilar and I would expect some reference to the previous literature in the discussion section.

Reference is made to the suggested reference (p5, line 10) indicating that the article discusses several important aspects of maternal immunization that were outside the scope of our project.

Methods: Its not clear how the list of priority areas was reduced from > 100 to 45 priorities apart from discussions being held at a meeting, further detail is required.

The planning team held a meeting at the end of the collaborative workshop to gather all the knowledge gaps identified and established a list of the 45 gaps that were deemed most relevant during the workshop. So, the selection of the 45 gaps for the online survey was based on the discussion held at the workshop. This is explained in the combined revised manuscript (p6, line 7 and Supplemental materials).

Introduction page 3 - last paragraph - it is suggested that the process outlined is faster than a systematic review - what evidence is there that this is true. A systematic review can be completed over several months.

We indicate in the Supplemental materials (Panel 1) the difference between a scoping review and a systematic review, how the nature of a scoping review can make it a faster process and the basis for the selection of this approach to reach the objectives of the project.

Overview - how were summaries prepared - was there a template, an example as a supplementary document should be provided. Overview section is quite long and could be better structured.

The summaries were prepared by the lead experts on the basis of the categories used to present the results of the scoping reviews (Supplemental Table 3 in Supplementa materials).

The Figure 1 included of the process is useful but needs more detail to help the reader better understand the components of the process.

The purpose of the figure is to represent graphically the flow of the process. We suggest that the content of its components are best explained by the text of the Supplemental materials. We therefore limited the information provided in the legend of the figure to the essential content of the components.

References - only 1 reference is given, I expect there to be some discussion of the findings in relation to other publications addressing the same topic

The combination of several manuscripts led us to include additional references on the topic. The single reference included in the supplemental materials is meant to provide more information on scoping reviews.

Scoping reviews followed by formal literature searches seems to duplicate the process.

We clarify in the Supplemental materials that the scoping review includes a literature review.

Did all experts complete their task or were some expert topics incompletely addressed?

Each expert completed their task to the best of their ability. The collaborative workshop was organized to further ensure the coverage of the knowledge and the identification of research gaps.

Last sentence page 4 - "thoughtful assessment by the expert" - is this the domain expert - please clarify

This is clarified in the Supplemental materials.

The scoping review process described in the second paragraph page 5, appears the same as a systematic review, not sure what the difference is?

As indicated above, the Supplemental materials clarifies the difference between a scoping and a systematic review.

Consultations and workshops interviewed leaders at major companies active in the field - which companies? were these vaccine manufacturers or other companies?

Was ethics approval obtained to conduct the interviews as part of this research?

We indicate that the consulted companies are vaccine manufacturers. We consider that their name is not essential information for the project and that not naming them is probably preferable.

Manuscript 3. Immunobiology of maternal immunization: a landscape analysis (THELANCETID-D-16-00250)

Reviewer #1:

This review timely addresses an important topic given the recent recrudescence of the interest for maternal immunization and the development of novel vaccines specifically for this purpose. It is well structured, although not always well balanced between the various sections. The quoting of specific studies and their interpretation is not always state-of-the-art, and the most relevant information not always included.

Specific comments to improve the manuscript include:

Pregnancy and immunoglobulins: to be informative the paragraph on IgG glycosylation should

- be more specific: describe changes in the Fab or the Fc fragments, increased galactosylation and sialylation versus reduced bisectoin, etc.
- describe the potential /theoretical impact of such changes on the function of antibodies for the mother and for the fetus, which is remarkably vague at this stage.

This information is included in the combined manuscript (p9, line 16 to p10, line 4).

Pregnancy and vaccine responses:

- This section is insufficiently documented, as authors entirely skipped a large body of evidence generated with tetanus toxoid vaccines ! Additional studies documenting vaccine responses during pregnancy can easily be retrieved for hepatitis B or more recently for GBS, including in Africa.

We indicate in the manuscript that our analysis was primarily focused on studies comparing vaccine responses in pregnant and non-pregnant women. To follow the suggestion of the Reviewer, we included some additional references to studies that did not include a non-pregnant control group and showed potent immunogenicity (p10, line 20)

- Ref 28 is misquoted: the authors of this small study did report (marginally) increased plasmablasts but NO significant differences for any influenza strain in postvaccination geometric mean HI or MN titers. That this study "suggested higher antibody responses" is thus not true and should be corrected.

We agree with the Reviewer that a more cautious interpretation of this article is more appropriate. We therefore deleted the comment on the antibody response.

- An important reference is missing re responses to pertussis immunization in pregnant or non-pregnant women: Halperin BA, Clin Inf Dis 2011, doi: 10.1093/cid/cir538

Our understanding is that this study involved women post-partum and not pregnant women. The upper limit of 150 references imposed a careful selection of the most important ones. We favored other references and did not include the one suggested by the Reviewer.

- The report from Abu Raya on the increased avidity following immunization early than late in the 3rd trimester should be contrasted by the lack of support in favor of this hypothesis by

Maertens K et al (Vaccine 2015, doi: 10.1093/cid/cir538)

We agree with the Reviewer that the letter by Maertens et al provides useful information. We included the reference and provide a more balanced view on this point (p11, lines 14 to 16).

- The report of Healy (ref 33) showed that similarly low titers were present at birth in neonates from mothers immunized before or during early (first trimester) pregnancy- compared to later (3rd trimester) immunization. How did the authors conclude from these findings that they "suggest that qualitative differences in the antibodies produced may affect placental transfer" ? This is consider to essentially reflect the fact that pertussis antibodies are only transiently increased in adults and the authors' interpretation should be revised.

We agree with the reviewer that our initial interpretation is not sufficiently supported by the data included in this article. We therefore deleted this part.

- Ref 33 is misplaced or should quote an official recommendation schedule, not a research manuscript.

The CDC recommendation is included (p11, line 23).

- Ref 34 is a major contribution which will lead to a change of the timing of maternal immunization, starting with the UK in April 1st, 2016. The authors should mention the hypotheses most likely to result into higher cord blood titers following 2nd than 3rd trimester immunization.

The reference is further emphasized and the most likely mechanism is mentioned (p12, lines 1 to 3).

- Ref 27 (antibody decay) would be better placed earlier in the paragraph, just after "relatively limited information is available on this point".

The structure of the paragraph has been modified according to the suggestion of the Reviewer (p11, lines 19 to 21).

- The paragraph on the potential influence of pregnancy on inflammatory responses is miserable: rather than extrapolating on correlations between cytokine responses and subjective symptoms in a small number of women, it should refer to the large body of evidence comparing adverse reactions in pregnant or non-pregnant women (for example following tetanus immunization).

As indicated in the introduction, a discussion of the safety of maternal immunization is beyond the scope of the project. Our aim was to mention that little is known about the innate/inflammatory responses to vaccines in pregnancy. This point is further clarified and reference is made to the generally good safety profile of vaccines in pregnant women, as suggested by the Reviewer (p 12, lines 4-7).

- Summary of the section: The authors are most speculative based on limited/small studies, whereas the main conclusion should be that vaccine responses elicited during pregnancy appear as remarkably similar to those elicited before or after pregnancy - at least on a quantitative basis.

The summary has been modified following the suggestion of the Reviewer.

Figure 1 :

- IgG titers : The authors construct their graph illustrating a candidate vaccine only eliciting transient (months) antibody responses. This should be
 - o Explicated in the legend
 - o Completed by a graph showing that vaccines inducing sustained responses (i.e. tetanus toxoid, measles, etc.) can readily be given before immunization as high antibodies persist throughout pregnancy
- Avidity : for their pregnancy immunization (blue line), the authors should explain that their graph only applies to a primary immunization (which requires time for avidity maturation) and not a booster strategy (such as pertussis or influenza) which readily reactivates high-affinity antibodies
- Transferability : I do not understand this graph -which is a copy-paste of that on IgG titers. Transferability should be 0 before pregnancy and shown to increase between the end of the first trimester and term. To delete or correct.

Because of the combination of the manuscripts recommended by the Editor, Figure 1 has been deleted. Although we consider that the concept of transferability of maternal antibodies, i.e. the potential of a given antibody to be transferred across the placenta at a given time during pregnancy, is of potential importance, we acknowledge the fact that it is not yet supported by clear data and can therefore be excluded from this review.

Transfer of maternal immunity through the placenta:

- IgG transfer and preterm birth: the authors fail to report the many studies which assessed vaccine antibodies in preterm versus term infants - whether these were induced by maternal immunization before or during pregnancy.

References are provided to support the discussion of the point raised by the Reviewer. Given the limitation of the number of references that can be included in the manuscript, we had to make a selection for this and other important points. We hope that our selection includes relevant and important publications.

- Factors influencing IgG transfer
 - o Ref 33 is against misused ("Studies suggested that the quality and structure of IgG may influence their transplacental transfer"), see above: nothing suggests to date that antibodies elicited at a given titers following immunization during pregnancy are more readily transferred.

As indicated above, we deleted sentences related to this concept.

- o An important missing reference : Simister NE. Vaccine. 2003 Jul 28;21(24):3365-9.

The reference is included (reference 57).

- o The discussion on the potential role of glycosylation on antibody transfer should be more balanced : ref 57 used much better techniques and should be quoted as concluding that the placental IgG transport is NOT Fc glycosylation selective.

A more balanced view of this point is provided, as suggested by the Reviewer.

- Summary : the design of optimal maternal vaccination strategies will not result from "defining the factors underlying the transferability of maternal IgG" : regardless of these factors, what counts is the magnitude and quality of antibodies present at delivery, whether premature or term. Please reformulate.

This summary has been re-formulated to emphasize the point stressed by the Reviewer.

Maternal immunization and infant immunity:

- "How long maternal antibodies persist above protective levels in the infant is a function of their half-life..." should be modified by "is a function of 1) the titers present at birth and 2) their half-life".

The sentence has been modified following the suggestion of the Reviewer.

- Clinical protection and correlate of protection: This paragraph is of little relevance in this review - and appropriately discussing CoP would require much more space / referencing. The focus of the authors on pertussis, GBS and RSV seems to be ad hoc (meeting report ? personal interest ?) - whereas clear CoP relevant for maternal immunization (tetanus for example) are not discussed. The discussion on the baboon model and lack of protection against colonization is misleading as it suggests that this is specific to maternal immunization whereas it is specific of aP vaccines (see Warfel JM PNAS 2014, doi: 10.1073/pnas.1314688110)

The paragraph has been deleted. On the other hand, the focus of the project on the four pathogens is explained in the manuscript. Discussion of pathogen-specific points have been reduced or deleted throughout the manuscript as they are discussed in the two companion articles that focus specifically on influenza/pertussis and RSV/GBS.

- Maternal antibody decay: how strong is the data concluding to differences in T1/2 between maternal antibodies of different antigen specificities ? Was it generated in the same women, comparing T1/2 of antibodies to tetanus or pertussis antigens ? If data is strong, could the authors elaborate on how antigen-specificity might affect antibody half-life - considered as essentially reflecting FcRn-mediated transfer ?

We agree with the Reviewer that this point lacked clarity, as T1/2 for antibodies of differing specificity have not been rigorously compared in the same subjects. This point is presented in a more careful way and potential determinants of antibody T1/2 are mentioned (p21, line 21 to p22, line 1).

- Interference with infant immunization:
 - o This is again a large topic which is difficult to summarize in a single paragraph. As such, the discussion is missing the mention of the key studies (including recent ones) and is not easy to follow... It should at least identify the factors that most critically influence interference (maternal titers at time of immunization, vaccine dose / antigen content (which is crucial to understand pertussis !), whether maternal antibodies target the protective antigen or its carrier (cf Ladhani SN Clin Infect Dis. 2015 doi: 10.1093/cid/civ695), and whether one considers the influence on primary or secondary responses).

We agree with the reviewer that interference is an important issue, difficult to summarize in a single paragraph. However, we do not understand the comment that we were missing key studies (e.g. the study mentioned by the reviewer as missing (Ladhani 2015) was our reference 89, now reference 149) or that we did not identify the most 'critical factors'

influencing interference, as discussion of all of the factors the reviewer mentioned as missing (maternal titres, dose, and antigen content) make up the bulk of that paragraph. We take the reviewer's hint that the paragraph could be written more clearly, and thus have restructured it.

o The conclusion of the authors that "feedback regulation via FcγRIIB appears most consistent with existing evidence" is abrupt as this hypothesis is difficult to reconcile with a number of observations / studies. It should be either amended or supported by the appropriate argumentation.

We agree with the Reviewer that this point remains unclear. We have therefore removed the reference to the FcγRIIB mechanism and we emphasize the gap in knowledge.

- Summary:

- ☐ This summary is remarkably vague and could be made more accurate
- ☐ Figure 2 is quite basic and not informative, bringing nothing more than what is in the text. Suggest to edit (i.e. with a detailed illustration of the influence of maternal antibodies on infant cellular responses) or delete.

We agree with the Reviewer that the figure did not include more information than the text and we deleted it. The summary has been rewritten to make it more accurate.

Transfer of maternal immunity through breastfeeding:

- This section would be better placed after that on "Transfer of maternal immunity through the placenta", to conclude with the more important section on "Maternal immunization and infant immunity"

The section has been moved as suggested by the Reviewer.

- In the absence of data relating vaccine-induced breast milk IgA, IgG or leucocytes to infant protection, this section is hypertrophic (was it perhaps copy-pasted from another document such as a grant preparation ?). It could be shortened - allowing much more space to be dedicated to the section on maternal immunization and infant immunity.

The paragraph has been shortened and we better highlighted what may be specific and critical to transfer of immunity through breast milk of immunized mothers.

- Summary : could be more factual rather than a call for active monitoring of what has not yet emerged as a key determinant of the impact of maternal immunization.

The summary has been modified following the suggestion of the Reviewer.

- Concluding remarks:

- To conclude to the existence of major knowledge gaps somehow implies that maternal immunization should be used with caution pending more information... which is likely not a reflect of the authors' position ?!

The concluding remarks have been rewritten to provide a more balanced view of maternal immunization and of the importance of identifying and filling knowledge gaps.

Table 1 is disappointingly vague. A suggestion would be to illustrate how the questions selected by the authors would inform the design of current / future maternal immunization strategies against tetanus, influenza, pertussis, RSV or GBS, for the most important ones.

Table 1 includes the knowledge gaps prioritized through the on-line survey and that are relevant to maternal immunization in general. Knowledge gaps specific to the four pathogens are listed in the Supplemental materials and the ones selected through the online survey are listed and discussed in the two companion articles.

Reviewer #2:

The authors have reviewed the literature to describe the current status of knowledge on the immunobiology of maternal immunization.

The authors present a comprehensive review of the literature, however, it is not clear that this review could be considered a "landscape analysis" considering that the methods of the systematic review required for a "landscape analysis" are not described. As such, the authors should consider either modifying the title of this review, or providing additional information on the methodology in the document.

The methodology used for the landscape analysis is detailed in the combined manuscript and the objective of the scoping (rather than systematic) review selected for the project is explained.

The article is well written and structured in a way that allows the reader to review important concepts revolving around the topic of maternal vaccination and maternal immune responses, and the format with a summary at the end of each section is quite helpful to bring up the most salient points to the reader. In general, the manuscript seems to highlight more aspects of what is not known about the immunologic responses during pregnancy, while it would be helpful to highlight as well what type of solid knowledge exists at this time. It is understood that the field of maternal immunization and the understanding of the immune function of pregnant women, the fetus, and the neonate are also evolving, yet readers count on the guidance of experts like the authors of this manuscript to provide a clear perspective of the current knowledge. I find the first section "impact of pregnancy on vaccine responses" as the most difficult to follow. Comments are provided to request for clarifications in different sections of the manuscript.

We thank the reviewer for this comment. The manuscript has been revised and combined following the recommendation of the Editor with two other manuscripts taking the comment into account and improving the content and structure of the section on the « impact of pregnancy on vaccine responses ». We would like to emphasize the fact that we had to respect wordcount and reference number limits and that we therefore had to select the most relevant and important information and references. We hope that our selection will appear appropriate to the Reviewers.

Comments specific to each section:

Impact of pregnancy on vaccine responses

Pregnancy and B lymphocytes. The first sentence in the first paragraph sets the expectation that this paragraph will describe what changes occur in the number and function of B lymphocytes in pregnancy. The paragraph does indicate that pregnancy is associated with decreased B cell numbers in peripheral blood, but it is not clear what the magnitude of this difference is or what the implications are. The paragraph then goes on to discuss studies in mice (have similar results

been described in humans?), and in healthy adults with HIV or other co-infections. It is not clear how this is relevant to changes seen in "normal" pregnancies. The last sentence then indicates that "few studies have suggested an impact of pregnancy on memory B cell subsets..." but what that impact is is not described. At the end of this paragraph, it is still not clear what functional changes occur in B lymphocytes during pregnancy.

Pregnancy and Immunoglobulins - It is understood that even though the number of B cells seem to be lower during pregnancy, these cells are able to increase the production of IgG, yet the levels of IgG are described to be lower in pregnant than in non-pregnant women, considered to be from hemodilution. Would this (hemodilution) be the same reason why B cells are lower too? If so, what is the significance of these findings?

The paragraph on B lymphocytes has been shortened and we have focused its content on the evidence that pregnancy is associated with changes in B lymphocyte number and functions. We further clarify the fact that animal studies indicate that reduced hemopoiesis is the most likely explanation for the reduced B cell number. We discuss further in the section the observation that vaccine responses appear quantitatively similar in pregnant and non-pregnant women whereas the impact of pregnancy on the quality of antibodies remains less characterized.

Regarding hyperglycosylation, given the changes described, can you comment on the potential impact of this change? It seems that it is a beneficial change, for the protection of the fetus; is there any evidence that this change is detrimental for the mother?

We further discuss the changes in IgG glycosylation, as suggested by Reviewer 1, and the potential consequences of these changes for the mother and newborn.

Pregnancy and antigen presenting cells - Please comment on the implications - of the higher mDC:pDC ratio that is described in pregnant women in this paragraph.

Changes in APCs associated with pregnancy are likely to be important for successful pregnancy but their impact on vaccine responses cannot be predicted at this stage. This is emphasized in the revised manuscript (p10, lines 13-15).

Pregnancy and vaccine responses - please clarify in the sentence regarding magnitude of antibody responses in women vs men if this is to any vaccine? or live vs. inactivated vaccines?

We now indicate that the difference applies to many vaccines and we provide a recent reference (reference 25) where this point is discussed in more details than we could do it in our manuscript.

At the end of this paragraph, you discuss that whether the gestational age of pregnancy affects responses to vaccines is uncertain, you do not cite some recent documents that provide insight on this aspect - see Abu Raya 2015 (pertussis vaccines) and other studies such as RSV vaccines and pneumococcal vaccines as reported in recent studies or older literature. Also, in this section, more recent data is available from the 2009 H1N1 studies performed in the US (Jackson and Patel) and Europe.

This point is further discussed and, as also suggested by Reviewer 1, includes references by Abu Raya (reference 41) and by Maertens (reference 42).

Second paragraph of page 5 - clarification, pertussis immunization is recommended in the

second or early third trimester, not just third trimester. Also, the reference cite (33) does not seem to be the most appropriate for the specific recommendation on pertussis vaccination (see CDC- ACIP recommendations form 2012 for US recommendations).

The reference has been deleted from the sentence and the CDC recommendation is included instead (p11, line 22).

Third paragraph in page 5 - there is mention in the last sentence of a "possibility of increased vaccine reactogenicity in pregnant women is contradictory to the previous data cited where pregnant women seem to have less reactivity/inflammatory activation in general compared to non-pregnant women. Lower reactogenicity has been documented with a number of vaccines given in pregnancy. Please clarify.

This point was also raised by Reviewer 1. As indicated in the introduction, a discussion of the safety of maternal immunization is beyond the scope of the project. Our aim was to mention that little is known about the innate/inflammatory responses to vaccines in pregnancy. This point is further clarified and reference is made to the generally good safety profile of vaccines in pregnant women, as suggested by Reviewers 1 and 2 (p12, lines 3-6).

page 6 - paragraph on transfer of antibodies - the last sentence discusses qualitative differences in the antibodies produced that may affect placental transfer, with specific mention to pertussis and the timing of immunization pre or during pregnancy, however, there is no mention of the effect of the concentration of antibodies on the rate of transfer - is this the "qualitative" difference that is described, is. the actual concentration of antibodies, which is likely a factor in the rate of transfer as is the time available to transfer?

These points have been clarified and, as suggested by Reviewer 1, we decreased the emphasis on the possibility that qualitative differences in maternal IgG, beyond subclasses, could influence transfer. Although we consider that this would be an interesting area to explore, we acknowledge the fact that the concept is currently not supported by data.

Influence of maternal factors on vaccine responses

Reference 36, is from 2011, does not include data from numerous more recent studies of vaccines in pregnancy.

Summary of this section - please indicate how "...pregnancy might impact" the different issues cited (ie. increase or decrease in these).

As also indicated in the reply to comments of Reviewer 1, our analysis was primarily focused on studies comparing vaccine responses in pregnant and non-pregnant women. To follow the suggestion of the Reviewers, we included some additional references to studies that did not include a non-pregnant control group and showed potent immunogenicity (p10, line 19).

Transfer of maternal immunity through the placenta

When discussing the differential IgG subtype production of antibodies by polysaccharide vaccines, note publication re. different IgG subtypes after maternal immunization with polysaccharide pneumococcal vaccines (munoz et al 2003, and others).

Additional information and references have been included following the suggestion of the

Reviewer (p14).

Last paragraph in this section, page 6 - please spell out PNG.

We thank the Reviewer for pointing this mistake.

Maternal Immunization and infant immunity

First paragraph - the last sentence implies that maternal immunization CAN have effects on the fetus and newborn infant beyond the passive protection. Please include references to support this statement. Is this effect known? What "effects" are impacted in the fetus and newborn? Please describe. If unknown, please modify this sentence to indicate that these potential effects are theoretical.

We provide a more balanced view on this point and we refer to the available evidence (p22 and 23).

Effectiveness of maternal immunization with pertussis in UK - new publications are available that should be included. See Ladhani study, and Miller.

The suggested reference is included (reference 146).

Maternal antibody decay "in infants" correct?

Correct. This has been clarified.

Interference with infant immunization

Reference 85 is related to infant vaccination not maternal.

The Reviewer correctly points this out. However, there is no data on maternal immunization and avidity. The reference we provided as #85 was in support that isotype and avidity is influenced by vaccination.

Also note, studies from Belgium and Vietnam (Maertens and Leuridan), and US (munoz) also discuss potential interference and these are not cited. Ladhani is cited.

The suggested references are included (references 37 and 50).

Please explain what is meant by "Feedback regulation via FcγRIIB ..." in the last sentence of this paragraph.

Following the comment of Reviewer 1, this sentence has been deleted.

Also, please discuss what the significance of "interference" as defined is, given that there seems to be no effect in priming or T cell responses.

We emphasize the limited understanding of the mechanism involved in interference and identify this as a knowledge gap.

Summary of this section, page 9. Again, please indicate what is meant by "maternal immunization may also affect the newborn in ways beyond providing protection via passive antibody". If possible be specific in describing what these "ways" are.

As indicated above, we further discuss and clarify this point (p22-23).

Transfer of maternal immunity through breastfeeding

Please note that there are other studies that have looked into breastmilk antibodies and potential effects on carriage of pathogens such as pneumococcus after maternal immunization. Would these studies warrant mention in this paragraph?

To our knowledge, only one study has demonstrated that maternal vaccination could increase prevention of infection in offspring by breastfeeding (Schlaudecker et al 2013). We have now added that maternal vaccination to influenza, pertussis, RSV, pneumococcal and meningococcal vaccine increased levels of specific IgA in breast milk and we had already mentioned impact of maternal vaccination to various pathogens on specific IgG levels. As suggested by Reviewer 3, we have also added a recent study indicating that magnitudes of HIV envelope specific IgA in breast milk of infected mothers are associated with a reduced risk of post-natal HIV-1 transmission (Polara et al 2016, reference 98) in addition to already referred observations observation on the role of milk IgG (Mabuka et al 2012, reference 109).

Section on breastmilk IgG - page 10.

Table 1 - listing the major gaps in the KNOWLEDGE of the immunobiology of maternal immunization is quite helpful, but also seems to be general. Would the authors, given their expertise, be able to describe in more detail some specific gaps related to maternal and infant immunity that should be addressed, in what priority, and how likely it is that such knowledge will be available in the near future? This could be commented in the text and/or in the table.

The gaps that have been identified by the experts during the consultative workshop are listed in the Supplemental materials. This list includes more gaps (general and pathogen-specific) than those included in and prioritized by the online survey.

Reviewer #3:

This review highlights the existence of many gaps in the immunobiology of "maternal immunization" crucial to understanding not only its protective role but also its modulatory effect on the development of immunity. The impacts of several factors, such as tolerance mechanisms, co-infections and microbiota, on the outcome of vaccination during pregnancy in low- and middle-income countries are not completely understood.

These factors are important considerations for current as well as future vaccines, particularly considering the burden of viral infections, such as Zika virus, that require public health intervention during pregnancy. The text contains relevant information and was described didactically in parts with an illustrative tone. Therefore, I have some concerns.

-Pregnancy and B lymphocyte

-The sentence should include other B cell populations: "Studies have examined the influence of pregnancy on B cells.... but also, others B cell populations: such as marginal B zone cells, transitional, germinal center B cell memory remain to be assessed.

Taking into account the limitation in wordcount, we clarified the point on memory B cells, following the suggestion of the Reviewer.

-Studies in mice indicated that estrogens produced during pregnancy reduce B cell lymphopoiesisThis section should mention other B cell populations with tolerance-inducing effects: and showing that B-1a B cells could be regulatory in animals suffering pregnancy disturbances but not from those developing normal pregnancies inducing the differentiation of naïve T cells into Th17 and Th1 cells (Muzzio DO et al 2014).

We thank the Reviewer for this interesting comment. However, we feel that the suggested point is too specific to be discussed under the format and within the scope of the project.

- This reference should also be included in this part of the text:
Pregnant women do not have impaired humoral immune responses to inactivated influenza vaccine and may have increased circulating plasmablast production compared to control women (Kay AW 2015)

The reference is included (reference 34) and a more balanced view of the results is provided, following the suggestion of Reviewer 1.

- Pregnancy and immunoglobulins.
- This part of the text could be included in the section addressing "the factors influencing IgG transfer" to avoid repetition.

We agree with the Reviewer on the importance of avoiding repetitions. However, we prefer to keep the two sections separate as they relate to different aspects of maternal immunization but we have taken care of avoiding repetitions.

-Pregnancy APC
-The absolute number of myeloid dendritic cells (mDC) ...This section could also include the reference Lee HR et al, 2014.
-In addition to the description of TLRs in APCs, some description regarding the inflammasome could be included (Maneta E et al, 2015) as a segue to the last sentence suggesting that the inflammatory milieu may alter APCs.

Given the limitation in reference number and the relatively lower importance of this section as compared to others, we did not include the suggested references, considering that the cited references provide sufficient information on these points.

- Although there is a decreased number of pDCs in the last trimester of pregnancy, their functional role is unknown because non-pregnant females have a basal level of IRF5 expression on pDCs, leading to higher IFN- α production upon TLR7 stimulation through oestrogen receptor 1 (Griesbeck M et al, 2015).

We thank the Reviewer for this suggestion. In order to provide broader information on this point, we include a recent review on sex differences in immune responses (reference 25).

-Pregnancy and Vaccine responses
Include a brief discussion of pregnancy outcomes after antepartum Tdap vaccination (Morgan JL 2015).

Our understanding is that the suggested reference is about safety aspects of antepartum

immunization. As safety was not the primary focus of our project, we prefer not to include the reference.

- Influence of maternal factors on vaccine responses.

The statement regarding malaria and placental integrity could include additional references: During maternal HIV infection or placental injuries, like those due to malaria, a large decrease in antibody transfer has been reported (Moraes-Pinto MI 1996, Farquhar C, 2005, Cumberland P, 2007, Palmeira P, 2012). One study determined that placental malaria or maternal HIV infection, independent of maternal hypergammaglobulinemia, affects the placental transfer of antibodies, and if the mother also has high IgG serum levels, placental transfer is even more impaired (de Moraes-Pinto, MI 1998).

We thank the Reviewer for these suggestions but we could not find the space to refer to these studies in our manuscript. The suggested points are covered in the manuscript using other references that we felt equally or more important.

-Transfer of maternal immunity through the placenta

- A classical description of the factors that influence the transfer of antibodies could address some updates on the subject.

We have further worked on this section to make it as updated as possible while keeping it accessible to a broad readership.

- The transfer of IgG4 and its specificity should be discussed.

We are not sure to have fully understood the suggestion of the Reviewer. We have included information on IgG3 transfer as this allowed us to further elaborate on the complexity of IgG transfer. We hope that this choice will meet the expectation of the Reviewer.

- The section should include the reference Bundhoo A 2015, which shows that human FcRn facilitates the transepithelial transport of IgE in the form of IgE anti-IgE ICs.

This reference is included (reference 58).

-Maternal immunization and infant immunity

Interference with infant immunization

Several mechanisms of negative interference ... include the reference: Edwards, KM, 2015.

Following the comments of Reviewers 1 and 2, we have decided to emphasize mechanism of interference as a knowledge gap without discussing the specific mechanisms that have been proposed.

-Breast milk IgA

The last paragraph should add the reference Pollara J, 2015, which shows that the magnitude of the breast milk IgA and secretory IgA responses against HIV-1 envelope proteins are associated with a reduced risk of postnatal HIV-1 transmission.

The reference is included (reference 98).

-Breast milk leucocytes

Add the following text: "Breast milk contains stem cells with multilineage properties (Hassiotou F, et al 2012)" as well as the work of Alsaweed M et al 2016, who show that regulatory biomolecules, including miRNAs, in human milk originate primarily from the mammary epithelium.

We agree with the Reviewer that stem cells and miRNAs in breast milk could be influenced by maternal vaccination and be involved in infant protection. However, currently, no studies have assessed the impact of maternal infection or immunization on these factors. We therefore prefer not to include the reference in the review.

Transfer of microbial antigens through breast milk

Prior to the description of the transfer of pathogens through breast milk, there should be a discussion of the work of W. Allan Walker 2015: the importance of breast milk influence on initial intestinal microbiota which prevents expression of immune-mediated diseases, underscoring the necessity of breastfeeding as the first source of nutrition.

Although very important, we consider that the suggested area is broad and goes beyond the scope of our review.

Maternal Immunization: Collaborating with Mother Nature

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Summary

Maternal immunization offers much hope to substantially reduce morbidity and mortality from infectious diseases after birth. The success of tetanus, influenza and pertussis immunization during pregnancy has led to consideration of additional maternal immunization strategies to prevent Group B Streptococcus (GBS) and respiratory syncytial virus (RSV) infections, among others. However, there remain multiple gaps in our knowledge regarding the immunobiology of maternal immunization that prevent optimal design and application of this successful public health intervention. An innovative landscape analysis was therefore undertaken to identify research priorities. Key topics were delineated through review of the published literature, consultation with vaccine developers and regulatory agencies, and a collaborative workshop gathering experts across several current maternal immunization initiatives - GBS, RSV, pertussis, and influenza. Finally, a global online survey prioritized the identified knowledge gaps based on expert opinion regarding their importance and relevance. This article presents the results of this worldwide landscape analysis and discusses the identified research gaps.

1 **Introduction**

2 Failure to improve survival in neonates by 2035 from the current status is estimated to lead to
3 116 million preventable stillbirths or neonatal deaths, 99 million survivors with disability,
4 and millions more with a lifelong increased risk for non-communicable diseases (1). The
5 underlying causes for the 2.6 million stillbirths per year are largely unknown, but
6 approximately 20% of the 2.9 million annual neonatal deaths are thought to be due to
7 infection (1). The transfer of antibodies from pregnant women to their offspring is profoundly
8 important for the health and survival of neonates and young infants, in particular by reducing
9 the risk of severe infections. Unfortunately, not all pregnant women have protective levels of
10 antibodies against pathogens affecting their offspring. The strategy of immunizing pregnant
11 women to enhance protection of young infants is rapidly gaining support from both the public
12 and health professionals alike (2). Contributors to this momentum include the global
13 reduction in neonatal tetanus as a result of maternal immunization, the benefits of seasonal
14 and pandemic influenza immunization for both mother and infant, and the positive impact of
15 immunization during pregnancy on recent pertussis outbreaks. These results are also
16 stimulating commercial development of new vaccines against additional threats such as group
17 B Streptococcus (GBS) and respiratory syncytial virus (RSV).

18 Recognizing the need to enhance the science of maternal immunization, the Bill and Melinda
19 Gates Foundation (BMGF) commissioned the authors to conduct a landscape analysis of the
20 immunobiology underpinning successful vaccination during pregnancy. The scope of the
21 review included all relevant immunobiological issues in general terms and as applied to
22 immunization against pertussis, influenza, GBS, and RSV specifically. The analysis also
23 aimed to identify differences that might be encountered among pregnant women in low and

1 middle income countries (LMICs) compared with high income countries (HICs) that may
2 affect the success of maternal immunization programs. An innovative approach was used to
3 rapidly identify and prioritize the current knowledge gaps in order to inform future studies.
4 This article describes the methodology and the results of this effort and discusses the
5 identified research gaps in immunobiology of maternal immunization that are generalizable
6 across pathogens. The research gaps specific to individual pathogens are discussed in two
7 companion articles. Other crucially important aspects of maternal immunization—safety,
8 public perception, and integration into existing global immunization programs—are outside
9 the scope of the project and will not be discussed here but are discussed in recent publication
10 summarizing the outcome of a series of meetings sponsored by the National Institute of
11 Health (3).

13 **Landscape Review Process and Knowledge Gap Prioritization**

14 To best capture the current state of knowledge, an innovative multi-stage review process was
15 undertaken. A detailed description of the methodology used and of the results of the analysis
16 is provided as Supplemental Materials. Briefly, an international team of 10 recognized
17 experts undertook a scoping review of the published English language literature since 2000.
18 The experts summarized the state of knowledge pertaining to their assigned area, including
19 their assessments of the gaps in understanding the biology of the immunization processes.
20 The team met at a collaborative workshop in Vancouver to share their assessments with 26
21 additional international experts who commented critically on the presentations (videos from
22 this meeting are available upon request from corresponding authors). Over 100 knowledge
23 gaps were identified through this process, attesting to the under-development of the

underlying science. To ensure that sufficiently broad deliberation was achieved and issues affecting translation addressed, further consultations were held with leaders of maternal vaccine development programs at 3 major vaccine companies and representatives of 2 major regulatory agencies (the US Food and Drug Administration and the European Medicines Agency) who freely shared their insights into the knowledge gaps and challenges.

To prioritize the identified knowledge gaps, topics considered most relevant during the collaborative workshop were included in an online survey completed by nearly 200 “content experts” from the global maternal immunization community. Respondents rated the importance of each knowledge gap; the results were remarkably consistent among respondents, including industry representatives, academic researchers, and national immunization policy makers. The top 20 knowledge gaps are listed in Table 1; each was rated ≥ 4 out of 5 (high to very high importance). To prepare the present and companion reviews, the authors integrated and summarized the information gathered from each of the above steps.

General Considerations Regarding Maternal Immunization Strategies

When considering the 4 disease targets for maternal immunization included in the landscape analysis, it is striking that no two are alike (Table 2), and that different strategies will likely be needed for each disease. All of which may make the production of a combined vaccine challenging. In order to focus on the immunobiology of maternal immunization, contextual differences, such as maternal disease risk, infant disease burden, global epidemiology, and microbial diversity will not be discussed further in this article. The common goal among maternal vaccination programs is temporary protection of the young infant against severe

1 illness and death by ensuring sufficient and timely transfer of protective antibodies from the
2 mother. This passive protection should persist until the infant is no longer at a high risk of
3 diseases (e.g. until 3 months of age for GBS disease) or until protection can be achieved by
4 active infant immunization (e.g. pertussis). Protection of the infant may also be achieved
5 indirectly by reducing carriage and/or disease in the mother, which subsequently reduces
6 transmission of pathogens to the infant (e.g. GBS, pertussis). Whether or not protection of the
7 mother against disease is also required is another important factor in determining the timing
8 of maternal immunization. In the case of influenza, for example, it may be that immunization
9 early during pregnancy would be favoured to protect both the pregnant woman and neonate.
10 Finally, there may be additional benefits of pre-pregnancy immunization, to prevent
11 infections which may have harmful effects on the developing fetus. It is important to note that
12 a substantial limitation in our understanding of optimal maternal immunization for any target
13 is the lack of defined correlates of protection for young infants. Without a validated measure
14 of protection it will be difficult to compare results of studies in different settings or to
15 improve vaccines or immunization regimens using serologic criteria.

16 Immunization during pregnancy relies on the capacity of the pregnant woman to mount
17 appropriate primary or secondary antibody responses, depending on whether the pathogen has
18 been encountered prior to pregnancy. The notion that pregnancy is associated with the
19 induction of a number of immunoregulatory mechanisms that are essential for the survival of
20 the fetus suggests that antibody responses to vaccines may be different in pregnant compared
21 with non-pregnant women. Vaccine responses may be further influenced by complications
22 affecting pregnant women, such as chronic infections. Optimal protection of the young infant
23 is considered to rely on the effective transfer of maternal immunity through the placenta and

the persistence of this passive immunity for the duration of infant exposure to the particular pathogen. Additional protection may be provided by transfer of immunity via breast milk. However, the relative contributions of breast milk and serum antibodies to infant protection will be difficult to define but important to understand, especially for infants born prematurely with limited transplacental transfer of antibodies. These passively transferred maternal immune factors can further influence active immunity induced in the infant by natural infection or immunization. Sixty-eight knowledge gaps with regards to the impact of pregnancy on vaccine responses, the transfer of maternal immunity to the infant, and on infant immunity were identified following the collaborative workshop (Supplemental Material). The top 10 of these knowledge gaps were considered most relevant in the on-line survey are presented in Table 1.

Impact of pregnancy on vaccine responses

Studies indicate that pregnancy influences B cells and antigen-presenting cells (APCs); the potential impact on follicular helper T cells has not been assessed at all.

Pregnancy and B lymphocytes

Estrogen and pregnancy reduce B cell lymphopoiesis in mice (4). Reduction in circulating B cells numbers have also been shown in pregnant women but the potential impact on antibody responses to primary immunization is unknown (5–7). Few studies have suggested an impact of pregnancy on memory B cell subsets but no consistent picture has yet emerged (8–10). In addition, the potential impact of pregnancy on other B cell subsets, including transitional or marginal zone B cells, remains to be assessed. In populations living in LMICs, chronic exposure to microbial antigens such as *Plasmodium falciparum* induces high frequencies of

1 circulating atypical memory B cells (8,9). As these memory cells have a reduced capacity to
2 produce immunoglobulins, their increased frequency may limit responses to recall
3 immunization in both pregnant and non-pregnant individuals living in LMICs.

4 ***Pregnancy and immunoglobulins***

5 Studies regarding the influence of hormones on B cell functions support the notion that
6 pregnancy may impact the production of immunoglobulins. Estrogen increases the production
7 of IgG by human B cells (11). In addition, activated human B cells upregulate the expression
8 of the prolactin receptor and prolactin further decreases the threshold of B cell activation
9 (12). In mice, estrogen also upregulates the expression of the activation-induced deaminase,
10 the enzyme that initiates somatic hypermutation and class switch recombination of
11 immunoglobulins (13). On the other hand, serum IgG levels have been found to be lower in
12 pregnant than in non-pregnant women in both LMIC and HIC settings (14,15). The
13 mechanism involved is unclear, but could, at least partly, be due to hemodilution. Pregnancy
14 is also associated with modifications in IgG glycosylation(16). IgG are glycoproteins
15 carrying N-glycans at both the Fc and Fab segments which modulate their effector functions
16 (17). In pregnancy, total IgG have increased sialylation and decreased N-acetylglucosamine
17 bisection of both Fc and Fab fragments and increased galactosylation of Fc fragments (16).
18 Although the functional consequences of Fab fragment glycosylation remain unclear,
19 sialylation and galactosylation of Fc fragments have been associated with decreased
20 inflammation and were suggested to be involved in the remission of rheumatoid arthritis
21 associated with pregnancy (18,19). The potential implications of the anti-inflammatory
22 properties of maternal IgG on immune homeostasis and anti-microbial defenses in the fetus
23 and newborn have not been determined. Surprisingly, IgG of different antigen specificity

1 have different glycosylation profiles and this profile is modified following recent antigen
2 exposure (20). Moreover, IgG glycosylation patterns are different in populations living in
3 HICs versus LMICs (20). Studies are needed to determine the impact of pregnancy on the
4 glycosylation and effector functions of vaccine-induced IgG.

5 ***Pregnancy and antigen-presenting cells***

6 Pregnancy is associated with changes in numbers and phenotype of APCs. The number of
7 myeloid dendritic cells (mDCs) increases in the first trimester of pregnancy and decreases as
8 pregnancy progresses to reach similar counts in the third trimester as in non-pregnant women
9 (21,22). On the other hand, the number of plasmacytoid (pDCs) is reduced during the third
10 trimester of pregnancy (23). mDC and pDC were shown to express higher levels of Toll-like
11 receptors in pregnant compared with non-pregnant women (24). A number of differences
12 exist between APC from females and males that are induced by sex hormones and could
13 therefore be relevant to pregnancy (25). Modifications of APC are likely to be important for
14 successful pregnancy but the potential impact on vaccine responses have not been
15 determined.

16 ***Pregnancy and vaccine response***

17 The impact of pregnancy and sex hormones on B cells and APC suggests a possible influence
18 on antibody responses to vaccines. This potential is indirectly supported by the observation
19 that the magnitude of antibody responses to many vaccines is often higher in females than in
20 males (25). Most studies of pregnant women that demonstrated potent vaccine
21 immunogenicity, however, did not include a comparison with non-pregnant women (26–29).
22 Few controlled studies have been conducted that generally involved only small study
23 populations. Some studies reported similar responses to seasonal influenza vaccines in

1 pregnant and non-pregnant women whereas others detected differences in titers or
2 seroconversion rates (30–34). Factors responsible for the discrepancies between studies may
3 include differences in tested vaccines and participant characteristics. Two controlled studies
4 conducted in HICs showed similar antibody responses to Tdap immunization in pregnant and
5 non-pregnant women while two other studies in LMICs reported no impact of pregnancy on
6 the response to tetanus immunization (35–38). The immunogenicity of a conjugated GBS
7 vaccine was recently studied in South Africa (39). Although the responses were not compared
8 between pregnant and non-pregnant women, the vaccine was immunogenic in both. Whether
9 the gestational stage of pregnancy affects responses to vaccines has not been extensively
10 studied. Similar antibody responses to seasonal and pandemic influenza vaccination were
11 observed throughout pregnancy in two studies while a trend towards higher seroconversion
12 rates with a seasonal influenza vaccine was seen during the third trimester in one study
13 (27,31,40). The impact of pregnancy on the quality of antibody response to vaccines remains
14 largely uncharacterized. Conflicting results on the avidity of antibodies following pertussis
15 immunization during early compared with late in pregnancy have been obtained in relatively
16 small scale studies (41,42).

17 The persistence of antibodies following maternal immunization will influence the optimal
18 timing of immunization and the requirement to repeat immunization during consecutive
19 pregnancies; however, relatively little information on this topic is available. Antibody decay
20 following immunization with adjuvanted pandemic influenza vaccine was similar in pregnant
21 and non-pregnant women (33). Pertussis immunization is currently recommended during the
22 second or early third trimester of pregnancy to achieve sufficiently high titers of antibodies
23 close to delivery (<http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6207a4.htm>). This

1 recommendation is challenged by a recent study showing higher titers of cord blood
2 antibodies following pertussis immunization during the second compared with the third
3 trimester of pregnancy, suggesting cumulative transfer of antibodies (43).

4 Innate immune responses following maternal immunization have not been explored. One
5 study reported similar plasma levels of inflammatory cytokines in pregnant and non-pregnant
6 women following seasonal influenza immunization. This is in line with the similar or even
7 lower reactogenicity observed in pregnant women following influenza immunization (44,45).

9 ***Influence of maternal factors on vaccine responses***

10 Most studies reported no significant effect of maternal age, parity, socioeconomic status or
11 body weight on antibody response to vaccines during pregnancy (46–48). But parity was
12 associated with reduced antibody responses to *H. influenzae* type b conjugate vaccine in The
13 Gambia and with higher responses to pertussis toxin in Belgium (49,50). This finding may be
14 particularly important in LMICs where high order multiparity is more common. Few studies
15 suggested a limited impact of nutrition on vaccine responses during pregnancy (51,52).
16 Whether obesity affects immune response to vaccination in pregnancy is poorly understood
17 as very obese women (BMI >30) are typically excluded from clinical trials. Relatively little
18 information is available regarding the possible differences in vaccine immunogenicity
19 between LMIC and HIC resulting from health conditions of the mother. One study reported
20 no impact of *P. falciparum* parasitemia at the time of immunization on antibody response to
21 tetanus toxoid (35). However, HIV infection impairs responses to vaccines. In South Africa,
22 pregnant women with HIV infection have lower seroconversion rates after seasonal influenza
23 vaccination compared with uninfected pregnant women but antibody half live and vaccine

efficacy are comparable between the two groups (53,54). HIV infection was also associated with lower immunogenicity of a glycoconjugate GBS vaccine in pregnant women in South Africa (55). The impact of helminth infection on vaccine responses during pregnancy has also not been systematically analyzed (56).

Summary

Overall, studies indicate that antibody responses to recall immunization are comparable between pregnant and non-pregnant women. Whether primary responses to new vaccines will be impacted by pregnancy is still unknown. Limited data suggest that pregnancy might impact avidity maturation, class switch, and glycosylation of vaccine-induced antibodies. With the exception of HIV infection, maternal factors influencing responses to vaccines have not been clearly identified.

Transfer of maternal immunity through the placenta

IgG transfer and preterm birth

IgG is the only antibody which is directly transferred across the placenta (57). Recent studies indicate that other maternal Ig can be transported to the fetus when complexed with IgG (58). IgG are actively transported through the placenta by the neonatal Fc receptor (FcRn), and possibly by additional receptors that have not yet been identified (59,60). The FcRn is expressed by syncytiotrophoblasts covering the surface of the chorionic villi and transports IgG by transcytosis into the fetal circulation. Although the FcRn is expressed and functional in the placenta from the first trimester, most of the antibody transfer occurs after 28 weeks gestation (61,62). Preterm birth is therefore an important factor limiting the transfer of

maternal immunity through the placenta and may affect the transport of IgG1 more than IgG2 (63–66).

Preterm birth occurs in 5% to 18% of pregnancies globally and is a leading contributor to infant morbidity and mortality. In a recent systematic analysis, over 60% of all preterm births were estimated to occur in sub-Saharan Africa and South Asia (over 9 million of approximately 15 million births per year globally) (67). At 28–33 weeks gestation, fetal-maternal antibody ratios are typically 0.5–0.6, compared with ≥ 1.0 at term. Thus transfer of maternal antibody could therefore afford some potential protection even in prematurely born newborns if their levels were elevated by prior immunization (66).

Factors influencing IgG transfer

The rate of IgG transfer through the placenta is influenced by several factors including IgG subclass, antigen-specificity, and chronic maternal infections. IgG subclasses are transcytosed at different rates, with IgG1 being most actively transferred, followed by IgG4, IgG3, and IgG2 (59,68,69). IgG3 allotypes have different affinity for FcRn and this results in differential transfer ratios (69). It is puzzling that antibodies of different antigen specificities are transported at different rates across the placenta, resulting in different maternal:cord blood antibody ratios (70–72). Reported cord blood:maternal ratios range as high as 1.9 for pertussis to as low as 0.7 for GBS, with influenza ranging from between at 0.7 to 1.0 depending on the study (26,53,73–75). These differences may be partly related to the differences in IgG subclass proportions, as protein antigens generally induce IgG1 and IgG3 subclasses while polysaccharide antigens induce mainly IgG2 antibodies, but this hypothesis has not been systematically examined (57,72). Whether or not the structure of maternal IgG influences placental transfer beyond subclasses has not been clearly established. Few studies

1 have suggested that high avidity antibodies may be transferred preferentially across the
2 placenta (76,77). Historical studies also suggested a preferential transfer of
3 hypergalactosylated IgG but this notion is not supported by a more recent study based on
4 more advanced technologies showing no impact of Fc galactosylation on transfer (78,79).
5 Chronic maternal infections and hypergammaglobulinemia have a profound impact on
6 maternal antibody transfer (66). Reduced transfer of IgG is observed in women with
7 hypergammaglobulinemia, a phenomenon that may be related to the saturation of FcRn (80–
8 82). Hypergammaglobulinemia and the denudation of syncytiotrophoblasts from chorionic
9 villi could also be involved in the reduced transfer of IgG associated with placental malaria
10 (66,81). A recent study in Papua New Guinea indicated an association between reduced
11 transfer of respiratory syncytial virus (RSV)-specific IgG with hypergammaglobulinemia but
12 not with placental malaria itself (83). Maternal HIV infection also results in a reduction of
13 maternal IgG transfer (82,84–86). Intriguingly, the impact of chronic maternal infections and
14 hypergammaglobulinemia appear to depend on the subclass and antigen-specificity of IgG. In
15 a study in South Africa, maternal HIV infection was associated with reduced transfer of
16 naturally acquired GBS-specific IgG1 but not IgG2 (85). In a study in The Gambia, maternal
17 hypergammaglobulinemia was found to be associated with impaired transfer of total IgG1
18 and IgG2, but not IgG3 and IgG4, and with a reduced transfer of IgG against pathogen but
19 not vaccine antigens (81).

20 **Summary**

21 Transfer of maternal antibodies through the placenta mostly occurs after 28 weeks gestation
22 and is limited by preterm delivery and by chronic maternal infections. Maternal
23 immunization could compensate for this reduced transfer but the timing of maternal

immunization and vaccine formulations will have to be optimized to achieve this objective. The basis for the variable maternal antibody transfer according to their antigen specificity remains poorly understood. Further studies are needed to determine the role of IgG subclass or other structural characteristics in this variability in maternal transport.

Transfer of maternal immunity through breastfeeding

The importance of breast milk in post-natal life is highlighted by the strong correlation between breastfeeding and the profound reduction of risks of infection and infection-related mortality in infancy (87,88). However, only one study assessed the role of breastfeeding in protection against an infectious pathogen following maternal immunization. In Bangladesh, exclusive breastfeeding was associated with fewer episodes of respiratory illness with fever in children born to mothers immunized against influenza during pregnancy (89). Prevention of infectious diseases by breastfeeding is thought to be due to the strengthening of gastrointestinal and respiratory mucosal immunity by: (1) improving the function of the epithelial barrier through breastmilk high content of growth factors; (2) transferring antimicrobial factors such as lactoferrin and lysozyme; and (3) transferring microbial antigen-specific immunity (Figure 1). Maternal immunization may thus modulate antigen-specific immune factors in breast milk and promote antigen-specific immune responses in infants.

Breast milk IgA

Breast milk secretory IgA (sIgA) antibodies are specific for an array of common intestinal and respiratory pathogens because the selective migration of B cells originating from the mucosal membranes to the mammary gland (90). Higher levels of sIgA should therefore be induced by mucosal as compared with systemic immunization, as observed following HIV

immunization of lactating Rhesus macaques (91). The antimicrobial properties of sIgA depend on: (1) the inhibition of pathogen adherence to and invasion of mucosal epithelia; (2) the neutralization of pathogens and toxins; (3) the transfer of antigens across the mucosal barrier and the stimulation of low level inflammation (reviewed in (92)). The latter mechanism has been mainly described in mice. Few studies in humans have demonstrated the transport of milk IgA into the circulation of breastfed mature and premature newborns (90,93,94). In LMIC where prematurity and gut mucosal inflammation are frequent, IgA transport to neonatal circulation may be increased and prolonged and could therefore be particularly beneficial. On the other hand, breast milk IgA may have a negative impact on the response to mucosal vaccines, but this finding remains controversial (95,96).

A number of studies showed increased levels of antigen-specific IgA in breast milk following maternal immunization against influenza, pertusis, RSV, *Streptococcus pneumoniae* and *Neisseria meningitidis* (reviewed in (97)). The amount of breast milk and magnitude of secretory IgA responses against a consensus HIV envelope protein were recently associated with the reduced risk of postnatal transmission of HIV in Malawi. This observation highlights the need for development of maternal vaccination strategies increasing HIV-1 envelope-specific breast milk IgA to reduce mother-to-child HIV transmission (98). Importantly, maternal conditions that are known to negatively impact transplacental transfer of IgG do not affect IgA transfer through breast milk. Prematurity increases the transfer of growth and immune factors, particularly IgA, in colostrum and milk (99,100). Furthermore, breast milk concentration of total and pathogen-specific IgA is not affected by maternal HIV infection or by malnutrition (101–104).

Breast milk IgG

Breast milk IgG originate from serum through FcRn transport and from milk resident B lymphocytes (105). Total breast milk IgG concentration is about 10% of IgA concentration but it tends to increase with duration of breastfeeding (100,106,107). Increased concentrations of antigen-specific IgG are detected in breast milk following immunization against RSV and pneumococcus and following natural infection with GBS, rotavirus, and HIV (96,108,109). Evidence of a protective role of breast milk IgG was demonstrated in studies on HIV infection, where IgG had higher neutralizing activity than IgA, mediated antibody-dependent cellular cytotoxicity, and were inversely correlated with the risk of HIV transmission (109). Breast milk IgG were also inversely correlated with cytomegalovirus (HCMV) load, suggesting a protective role against HCMV transmission (110). However, the role of breast milk IgG in the defense against other pathogens has not been studied. Mouse experiments indicate that breast milk IgG can cross the gut barrier through FcRn and can thereby promote the transport of IgG-antigen immune complexes and stimulate immune response to antigens and pathogens (60,111–114). Whether this process occurs in humans is unknown.

Breast milk leucocytes

Breast milk contains neutrophils, macrophages, and lymphocytes (115). Common infections increase the number of total leucocytes in breast milk but whether similar changes occur post-immunization is unknown (116). Breast milk B lymphocytes are IgG producing memory cells. Their antigen-specificity was demonstrated in the context of HIV infection (105). Similarly, HIV-specific CD4 and CD8 T lymphocytes were detected in breast milk and may contribute to virus control through inflammatory cytokines and cytotoxicity (117,118).

1 Studies suggest that breast milk CD4 T cells may be transferred to human neonates and
2 induce transient specific cellular immunity (93,119,120).

3 ***Transfer of microbial antigens through breast milk***

4 Although pathogens can be detected in breast milk following maternal infection, transmission
5 to the offspring is not commonly observed, with notable exceptions including HIV, HCMV,
6 and HTLV-1 (121). The evidence suggests that breast milk immunity may prevent pathogen
7 transmission. In addition, studies indicate that exposure to pathogens through breast milk
8 induces immune responses in infants independently of transmission. Exposure to HIV-
9 containing breast milk is associated with the induction of mucosal IgG and IgA responses and
10 with systemic cell-mediated immune responses in uninfected infants (102,122). Similarly,
11 *Vibrio cholera* can be transferred through breast milk and induce either disease or
12 colonization associated with specific IgG responses in infants (123). These observations
13 suggest that breastfeeding can promote immunity to pathogens in infants by transmitting
14 pathogens that are attenuated by maternal immune responses and/or transfer of pathogen
15 antigens. Studies indicate that a similar process occurs following immunization of lactating
16 women with the live attenuated rubella vaccine (reviewed in (124)). Mouse studies have
17 shown that the intrinsic adjuvant properties of antigens, the level of IgG and vitamin A in
18 breast milk are critical factors in the induction of effector immune responses in the offspring
19 (125).

20 ***Summary***

21 There is strong evidence that breast milk is essential for mucosal immunity in infants and that
22 maternal vaccination increases antigen-specific immune effectors in breast milk. Mouse and
23 human studies further suggest that the transfer of microbes through breast milk may promote

active immunization in infants. Breast milk transfer of immunity by immunized mothers may be particularly relevant in LMIC where transplacental transfer of immunity is reduced by chronic maternal infections and the high rate of pre-term delivery. However, there currently exists little data linking breast milk immunity induced by vaccines and infant protection.

Maternal immunization and infant immunity

Following transfer across the placenta, maternal antibodies are expected to protect the infant from disease. However, a certain level of antibody (the presumed correlate of protection) has to be reached to provide clinical protection and this level needs to be maintained until the infant is no longer at risk, or is protected by active immunization. How long maternal antibodies persist above the protective levels in the infant is a function of the concentration of the antibody in the newborn at birth and the antibody half-life ($T_{1/2}$). Thus, the transplacental transfer and decay kinetics of maternal IgG in the infant are key determinants of the duration of protection. However, high levels of maternal antibodies present at the time of infant vaccination may also interfere with the immune response of the infant to the respective vaccine. Lastly, maternal immunization can have effects on the fetus and newborn infant beyond passive protection.

Prevention of infection and disease

The distribution of serum antibodies beyond the bloodstream of the neonate/infant is not well defined, but could limit what is achievable in terms of mucosal protection. For example, very little IgG is detectable in saliva of young infants until the teeth erupt (126), making sterilizing immunity against respiratory pathogens unlikely. A more readily achievable objective would then be the minimization of invasive disease severity rather than prevention of portal of entry

infection/colonization. This limitation is illustrated by the failure of various preparations of pertussis immune globulin to prevent colonization (and subsequent invasive infection) in humans and animal models (127–129). The recently observed effectiveness of maternal pertussis immunization in preventing infant disease represents an important advancement (130). If the benefit is largely attributable to minimization of disease severity such encounters could result in passive-active immunity, with active immunity following attenuated natural infection (131).

Maternal antibody decay in infants

The $T_{1/2}$ of IgG differs by subclass and is not a fixed entity but is directly proportional to the total IgG concentration; this is called the *concentration-catabolism effect*, where IgG catabolism is accelerated in subjects with increased IgG levels and conversely, reduced in subjects with a low serum IgG concentration (132). The molecular mechanisms underlying the differences in $T_{1/2}$ of the various IgG subclasses as well as the concentration-catabolism effect center around FcRn (59,60). Subclass and structural modifications of IgG have profound impact on the interaction with FcRn, and thus $T_{1/2}$. For example, IgG3 allotypes have different affinity for the FcRn and this results in different $T_{1/2}$ (69). Also, aglycosylated human IgG1 has a significantly shorter $T_{1/2}$ (62 h) than the glycosylated form (153 h) (132). As indicated above, glycosylation of maternal antibodies is modified during pregnancy (16,133), but how this relates to $T_{1/2}$ in the infant is currently not known. Furthermore, studies suggest that the $T_{1/2}$ of IgG in infants varies depending on the antigen-specificity of the antibodies as well as between populations. For example, reported $T_{1/2}$ in the infant of maternal antibodies specific for pertussis antigens is ~30-40 days, for tetanus ~50 days, but for GBS ~60 days (29,134,135). $T_{1/2}$ of maternal antibodies of a given specificity can also

1 vary substantially between populations; whether this variability involves differences in IgG
2 subclass or other structural differences has not been delineated (136–138).

3 ***Interference with infant immunization***

4 The presence of maternal antibodies to a particular vaccine antigen has been reported to
5 reduce antibody generation following vaccination of the infant with the same antigen
6 (reviewed in (139–141)). This is called *interference*. Maternal antibodies not only affect
7 levels of antibodies produced by the infant, but also can influence their quality (strength of
8 antigen binding or avidity) (141,142). Priming of T cell responses to vaccines does not appear
9 to be affected by passive antibodies and this probably contributes to the good response to
10 booster doses (139,140). The key factors influencing interference are antigen-specific
11 maternal antibody titers at time of infant immunization, as well as infant vaccine antigen-
12 content (including dose). For pertussis, maternally derived antibodies have been shown to
13 interfere with antibody responses with whole-cell vaccines, but less so when acellular
14 vaccines were used in the infant (37,50,143–147). Whether the improved response to
15 acellular vs. whole-cell vaccine among those with higher antecedent PT titers is due to higher
16 antigen load in the acellular product or to the absence of other components of the whole cell
17 vaccine lacking in the acellular product has not been determined (148). Given that the current
18 lead candidates for a maternal GBS vaccine are TT- or CRM197-conjugate polysaccharide
19 vaccines, it is worth noting that infants born to mothers with high anti-TT titers immunized
20 with Hib-T-conjugates have reduced anti-GBS responses but infants immunized with HbOC
21 (CRM₁₉₇) showed no interference (149–151). Although several mechanisms have been
22 proposed, the molecular and cellular basis of the interference remains incompletely
23 understood (139,140).

Influence of maternal immunization on infant beyond passive immunity

Following influenza (TIV) vaccination during pregnancy, anti-HA and anti-matrix protein IgM antibodies could be detected in 38.5% and 40.0%, respectively, of cord blood specimens (152). Given that IgM does not cross the placenta, this would be indicative of an active adaptive B cell response in the fetus. This was further corroborated by the detection of HA-specific T cell responses in some newborns of immunized women using synthetic peptide-HLA multimers. Similarly, earlier studies of tetanus vaccination during pregnancy reported detection of anti-toxoid IgM in sera of some infants (153,154). Furthermore, given that vaccines can have immune modulatory effects in postnatal life beyond initiating antigen-specific adaptive responses, i.e. non-specific effects (NSE) (155), it is conceivable that immunization during pregnancy could also have NSE not only in the mother, but also in the fetus and/or newborn. To our knowledge, this has not been systematically investigated. However, MF59-adjuvanted influenza vaccination during pregnancy led to an altered cytokine production profile in the nasal mucosa of 4 week old infants contrasting infants from vaccinated vs. unvaccinated mothers (156). The clinical relevance of either of these ‘unexpected’ findings (active *in utero* immune response; non-specific effects on the newborn after maternal immunization) is currently not clear.

Summary

Immunobiological parameters such as correlates of protection based on passively acquired antibody levels and half-life of the antibody are key determinants of the efficacy of maternal immunization. However, little is known about either aspect. Higher maternal antibody levels in the infant can interfere with the infant’s response to immunization; neither the mechanisms involved nor the relevance of this for protection have been determined. Finally, maternal

immunization may also prime immune responses in the fetus and thereby influence responses after birth.

Concluding remarks

The passive transfer of maternal immunity is considered central to anti-microbial defenses in early life (Figure 2). The proposed mechanisms center around active transport of maternal IgG through the placenta providing systemic immunity during the first months after birth until the infant actively acquires immunity through exposure to pathogens or vaccines. The immune components of breast milk can provide longer-term immunity at the mucosal level and could also contribute to the development of infant immunity at the systemic level.

Although maternal immunization is an effective strategy to increase anti-microbial immunity in early life, many knowledge gaps remain in our understanding of vaccine responses during pregnancy, the transfer and persistence of maternal immunity in infants, and the interactions between maternal antibodies and the infant immune system. This landscape analysis prioritized gaps that are of particular relevance to the development of new vaccines for pregnant women and to the implementation of maternal immunization worldwide (Table 1). Filling those gaps offers the potential to further improve this important public health intervention. This will require immunological studies of existing vaccines administered to pregnant women and the inclusion of immunological endpoints in the clinical studies of vaccines that are under development.

Contributors Statement

AM, DWS and TRK developed and managed the landscape analysis and synthesized the information. AM, VV, LP and TRK led the literature review on the immunobiology of maternal immunization. MG and GB provided major administrative support and participated in the synthesis of the information. AM, MS, ND, VV, LP, CEJ, SAH, KME, PH, PO, DWS and TRK contributed to the literature review and synthesis. AM, MS, VV, MG, DWS and TRK drafted the initial manuscript and all authors contributed to the final version of the manuscript.

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Table 1. Global Experts Survey: Top 20 Knowledge Gaps

	Likert Rating score* (maximum score 5.0)
1. Immunization During Pregnancy	
a) Impact of the type of vaccine antigen on maternal responses	4.1
b) Impact of health conditions on maternal immune responses	4.2
2. Transplacental Transfer of Antibodies	
a) Impact of timing of vaccination during pregnancy on net transfer	4.4
b) Impact of antigen type on maternal responses and transferability	4.1
c) Impact of pregnancy complications on antibody transfer	4.0
3. Protection of fetus and newborn infant	
a) Impact of maternal immunization regimen on cord titers	4.3
b) Impact of maternal immunization regimen on infant responses	4.3
c) Clinical relevance of interference with active immunization	4.3
d) Impact of maternal antibodies on effector and memory B cell responses of infants	4.0
e) Modulation of breast milk immune components by immunization	4.2
4. Pertussis vaccination	
a) Correlates of protection against colonization, disease, death	4.4
b) Requirement for multiple pertussis antigens, role of P toxin	4.2
c) Reactogenicity of repeated doses of Tdap in sequential pregnancies	4.0
5. Group B streptococcal vaccine	
a) Correlates of protection against colonization, disease, outcomes	4.5
b) Serotype specific immunogenicity, transfer and protection	4.3
c) Impact of serotype on correlates of protection	4.0
d) Effect of carrier proteins on responses of infants to vaccination	4.0
6. Respiratory syncytial virus vaccine	
a) Correlates of protection against infant disease, death	4.6

b) Protection against lower respiratory infection, disease	4.6
c) Impact of pre-existing immunity on maternal responses	4.0

*Rating score 4 = high importance, 5 = very high importance, on a 5 point Likert scale

Table 2. Maternal Immunization Landscape: No Two Programs are Alike

Consideration	Pertussis	Influenza	GBS	RSV
Maternal disease risk	+	+++	++	+
Infant mortality	++	+	+++	++
Infant disease frequency	+ (cyclic ¹)	++	+	+++
Disease seasonality	✓	✓	✗	✓
Microbial diversity	+	++	++	+
Licensed vaccine available	✓	✓	✗	✗
Maternal booster response expected ²	✓	Quasi ³	Not assumed	✓
Passive protection of infant	✓	✓	✓	✓
Maternal:cord Ab ratio	1.1-1.9	0.7-1.0	0.7-0.8	1.0
Antibody half-life (days)	36-40	40-50	30-44	36-79
Infant vaccination	✓	≥6 months	✗	(✓) ⁴
Correlate of protection	✗	Quasi ⁵	✗	✗
Functional immunoassay	✗	✓	? ⁶	✓
Competing control option	✗	✗	✓ ⁷	✓ ⁸

¹Increased disease incidence usually occurs every 3-4 years

²Via previous vaccination and/or infection

³Prior vaccination and/or infection will lead to partial protection due to virus evolution

⁴Monoclonal antibody administered to high risk infants during RSV season

⁵Correlates of protection based on hemagglutinin inhibition assay or microneutralization titers have not been validated in young infants and are not based on maternal immunization

⁶Bacterial killing in an opsonophagocytic assay has been suggested as a possible correlate of protection

⁷Intrapartum antibiotic prophylaxis has reduced the incidence of early onset GBS neonatal sepsis

⁸Monoclonal antibodies administered to high risk infants during RSV season reduces rates of hospital admission