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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.

## Introduction

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

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

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

## Landscape Review Process and Knowledge Gap Prioritization

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

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

Immunization during pregnancy relies on the capacity of the pregnant woman to mount appropriate primary or secondary antibody responses, depending on whether the pathogen has been encountered prior to pregnancy. The notion that pregnancy is associated with the induction of a number of immunoregulatory mechanisms that are essential for the survival of the fetus suggests that antibody responses to vaccines may be different in pregnant compared with non-pregnant women. Vaccine responses may be further influenced by complications affecting pregnant women, such as chronic infections. Optimal protection of the young infant 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

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

***Pregnancy and immunoglobulins***

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

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

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

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

***Pregnancy and vaccine response***

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

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

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

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

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

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

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

have suggested that high avidity antibodies may be transferred preferentially across the placenta (76,77). Historical studies also suggested a preferential transfer of hypergalactosylated IgG but this notion is not supported by a more recent study based on more advanced technologies showing no impact of Fc galactosylation on transfer (78,79).

Chronic maternal infections and hypergammaglobulinemia have a profound impact on maternal antibody transfer (66). Reduced transfer of IgG is observed in women with hypergammaglobulinemia, a phenomenon that may be related to the saturation of FcRn (80–82). Hypergammaglobulinemia and the denudation of syncytiotrophoblasts from chorionic villi could also be involved in the reduced transfer of IgG associated with placental malaria (66,81). A recent study in Papua New Guinea indicated an association between reduced transfer of respiratory syncytial virus (RSV)-specific IgG with hypergammaglobulinemia but not with placental malaria itself (83). Maternal HIV infection also results in a reduction of maternal IgG transfer (82,84–86). Intriguingly, the impact of chronic maternal infections and hypergammaglobulinemia appear to depend on the subclass and antigen-specificity of IgG. In a study in South Africa, maternal HIV infection was associated with reduced transfer of naturally acquired GBS-specific IgG1 but not IgG2 (85). In a study in The Gambia, maternal hypergammaglobulinemia was found to be associated with impaired transfer of total IgG1 and IgG2, but not IgG3 and IgG4, and with a reduced transfer of IgG against pathogen but not vaccine antigens (81).

***Summary***

Transfer of maternal antibodies through the placenta mostly occurs after 28 weeks gestation and is limited by preterm delivery and by chronic maternal infections. Maternal 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).

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

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

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

***Summary***

There is strong evidence that breast milk is essential for mucosal immunity in infants and that maternal vaccination increases antigen-specific immune effectors in breast milk. Mouse and 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

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

***Interference with infant immunization***

The presence of maternal antibodies to a particular vaccine antigen has been reported to reduce antibody generation following vaccination of the infant with the same antigen (reviewed in (139–141)). This is called *interference*. Maternal antibodies not only affect levels of antibodies produced by the infant, but also can influence their quality (strength of antigen binding or avidity) (141,142). Priming of T cell responses to vaccines does not appear to be affected by passive antibodies and this probably contributes to the good response to booster doses (139,140). The key factors influencing interference are antigen-specific maternal antibody titers at time of infant immunization, as well as infant vaccine antigen-content (including dose). For pertussis, maternally derived antibodies have been shown to interfere with antibody responses with whole-cell vaccines, but less so when acellular vaccines were used in the infant (37,50,143–147). Whether the improved response to acellular vs. whole-cell vaccine among those with higher antecedent PT titers is due to higher antigen load in the acellular product or to the absence of other components of the whole cell vaccine lacking in the acellular product has not been determined (148). Given that the current lead candidates for a maternal GBS vaccine are TT- or CRM197-conjugate polysaccharide vaccines, it is worth noting that infants born to mothers with high anti-TT titers immunized with Hib-T-conjugates have reduced anti-GBS responses but infants immunized with HbOC ($CRM_{197}$) showed no interference (149–151). Although several mechanisms have been proposed, the molecular and cellular basis of the interference remains incompletely 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] |

[1]Increased disease incidence usually occurs every 3-4 years
[2]Via previous vaccination and/or infection
[3]Prior vaccination and/or infection will lead to partial protection due to virus evolution
[4]Monoclonal antibody administered to high risk infants during RSV season
[5]Correlates of protection based on hemagglutinin inhibition assay or microneutralization titers have not been validated in young infants and are not based on maternal immunization
[6]Bacterial killing in an opsonophagocytic assay has been suggested as a possible correlate of protection
[7]Intrapartum antibiotic prophylaxis has reduced the incidence of early onset GBS neonatal sepsis
[8]Monoclonal antibodies administered to high risk infants during RSV season reduces rates of hospital admission

Figures 1 and 2

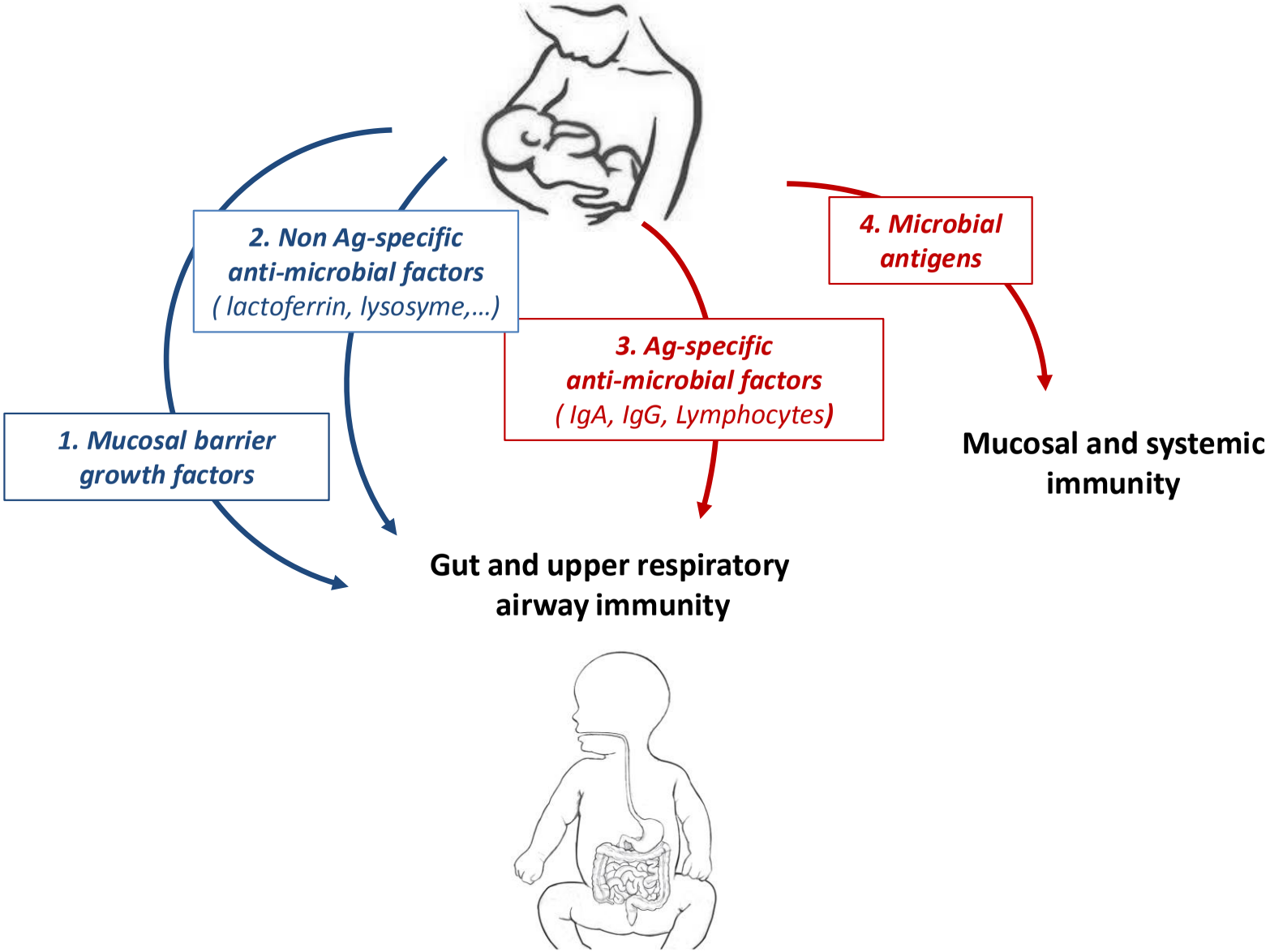



**Figure 1. Transfer of maternal immunity through breastfeeding.** Microbe-nonspecific immunity (blue) is promoted by breast milk through (1) growth factors improving the function of the epithelial barrier and (2) anti-microbial molecules. Microbe-specific immunity (red) is provided by Ag-specific maternal 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.

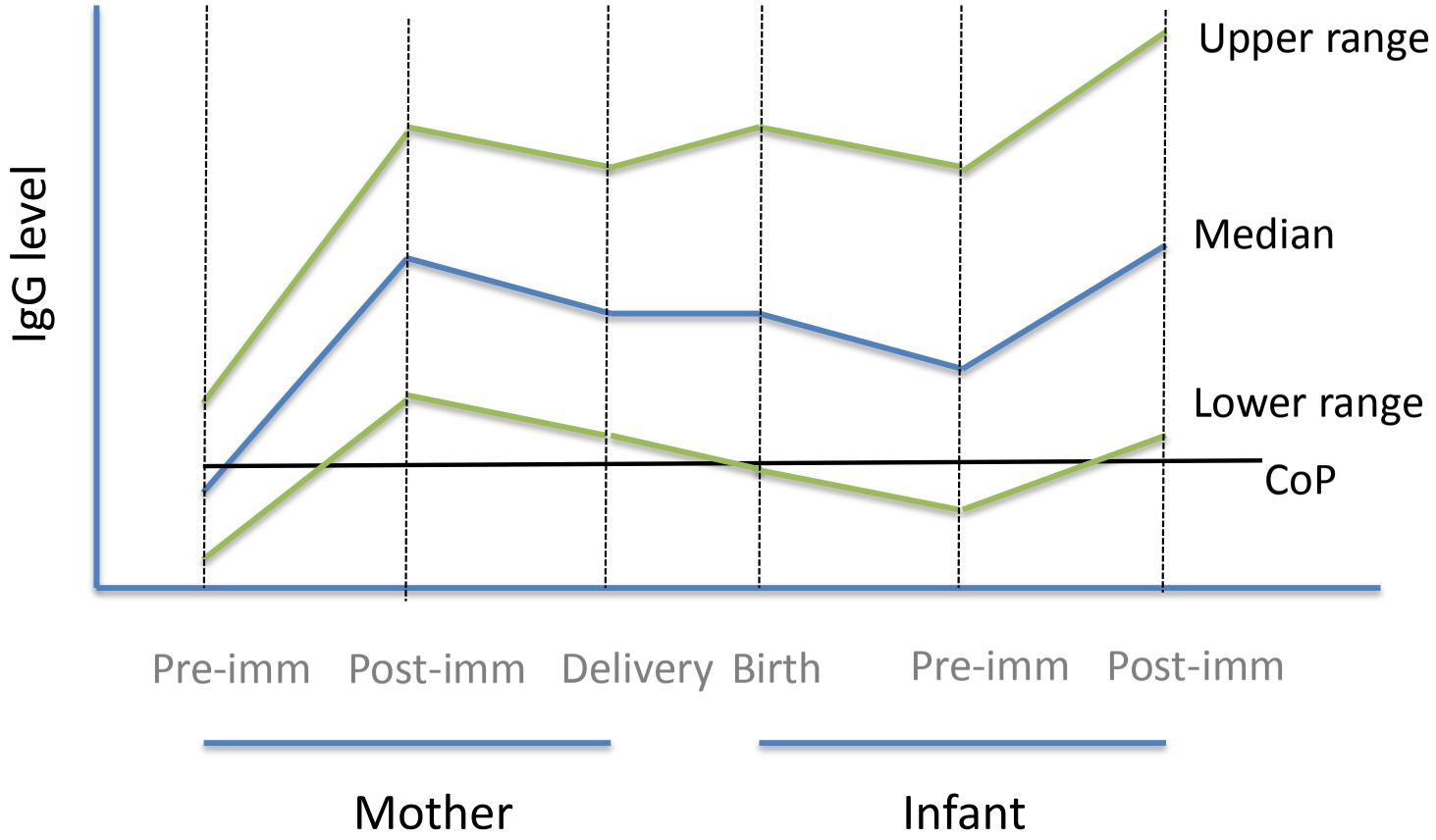



**Figure 2. Influence of maternal immunization on infant IgG before and after vaccination.** The horizontal black line represents a putative correlate of protection (CoP) for the disease of interest. The blue line represents median pathogen-specific IgG, and the green lines show the upper and lower limits of the potential IgG range. In the absence 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, Syndney, 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.

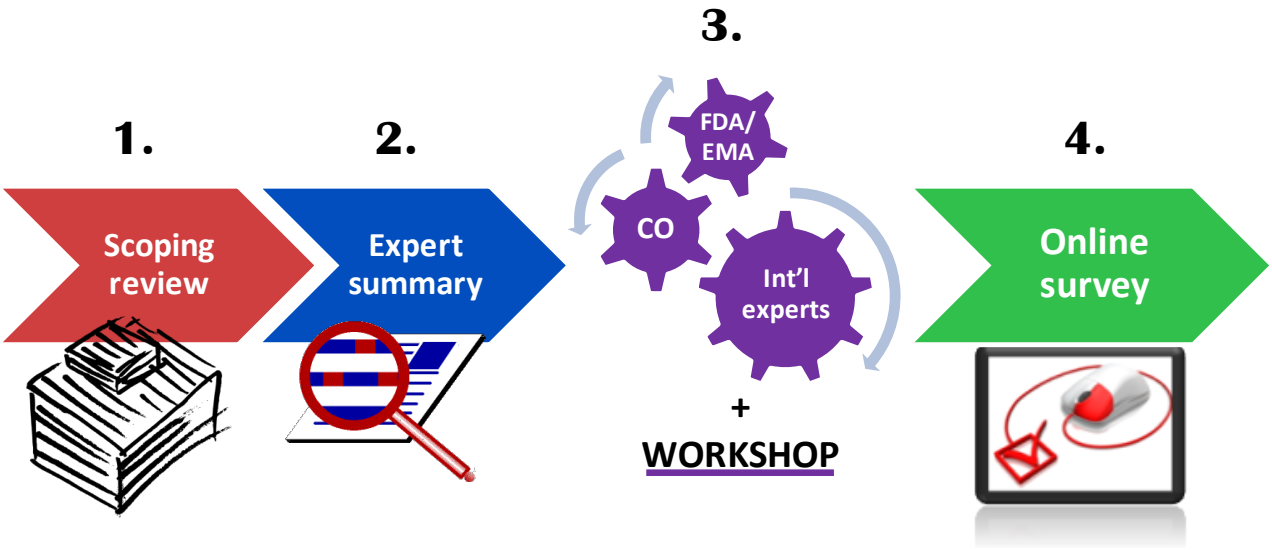
***Conclusion***

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

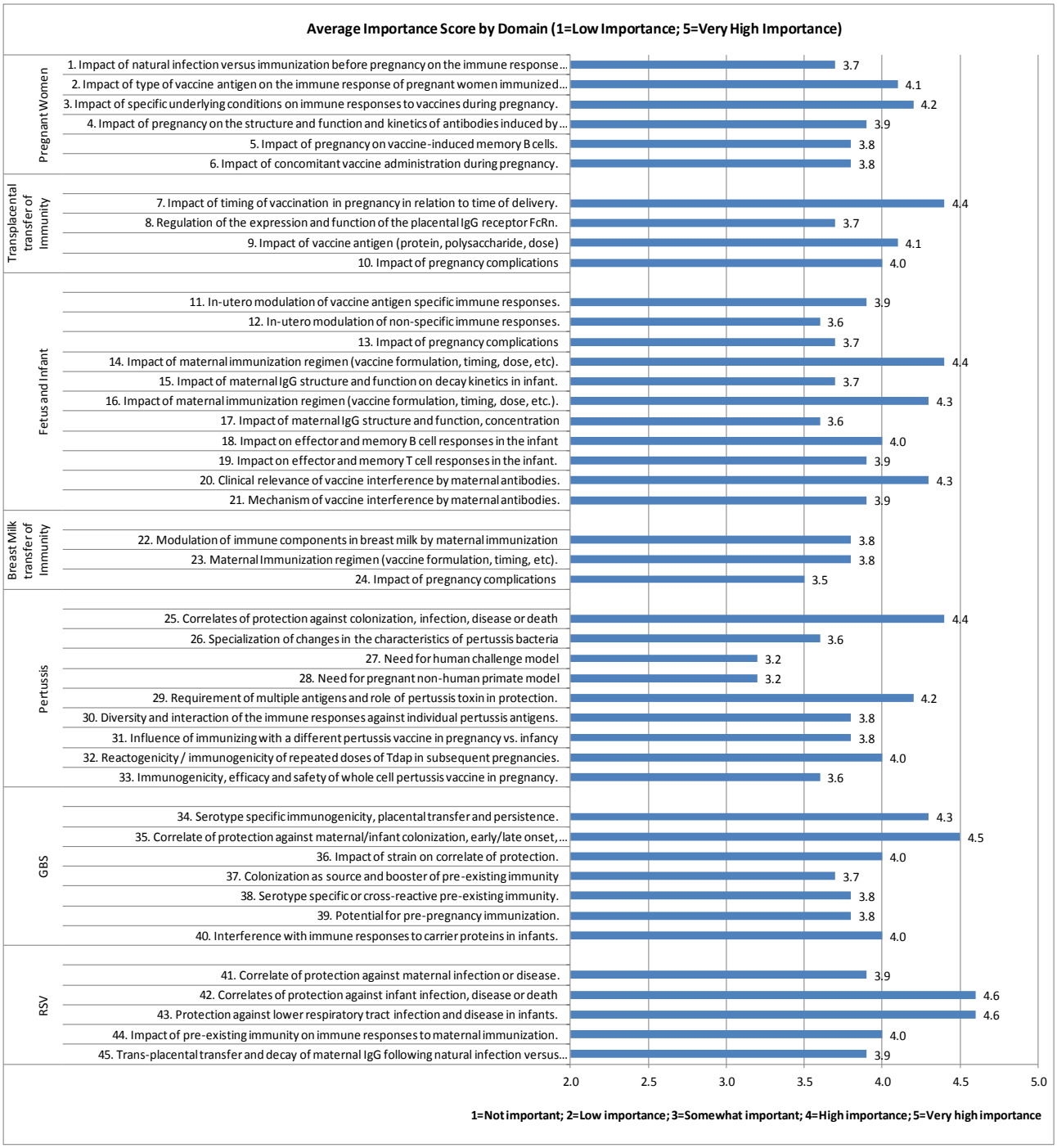
together, for the first time, experts across a wide range of disciplines relevant to maternal immunization. This unique amalgamation of individuals sharing a common interest and passion led to the natural and spontaneous formation of a global consortium of volunteers focused on advancing effective and 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 decayor 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 syncitiotrophoblasts
    - 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