Sex-specific endocrine and immune responses are widely recognized to account for differential disease outcomes between females and males. Surprisingly, sex-specific risk assessments for influenza, a viral pathogen that affects human populations worldwide through seasonal epidemics and irregular occurring pandemics, are sparse and—if available—ambiguous. To date, this precludes proposing an unequivocal sex-dependent susceptibility to influenza. However, one undisputable observation recurrently confirmed during influenza seasons of the last decades is the significantly increased risk for pregnant women. This increased risk is likely attributable to the contradictory demands for the maternal immune system to adapt to pregnancy and to simultaneously mount an immune response to clear the influenza virus infection. Here, we review published evidence on the potential association between sex on influenza risk and propose that future epidemiologic studies should carefully dissect surveillance data for sex-specific effects. Moreover, we propose potential mechanisms involved in enhanced risk for severe influenza during pregnancy that could be studied to identify causal pathways.

**Keywords.** influenza; sex; gender; immunity; pregnancy.
females has been attributed to the cellular mosaicism of genes encoded on the X chromosome. Females inherit 1 maternal and 1 paternal X chromosome, and the duplicate gene requires silencing to avoid double protein generation [8]. Because this gene inactivation occurs randomly and is not restricted to the maternal- or paternal-derived X chromosomes, possible X chromosome-linked mutations can be selectively silenced and the respective gene on the second X chromosome still provides the backup in females, which is missing in males. Of note, genes highly relevant for the recognition of viral pathogens, for example, pattern recognition receptors, are encoded on the X chromosome [12, 13].

Influenza is known to severely affect human populations worldwide through seasonal epidemics, pandemics, and localized outbreaks and is associated with high fatality rates. Individuals at high risk to develop serious complications from influenza infection identified to date include the elderly, young children, and individuals with preexisting medical conditions. Surprisingly, sex-specific risk assessments for seasonal, avian, or pandemic influenza, as well as in-depth understanding of associated biological pathways, are still elusive. Epidemiologic studies describing previous influenza seasons largely focus on virus characterization, severity of the infection, hospitalization rates, and deaths due to influenza-like illnesses by state and country. In the present review, we aim to summarize insights on sex-specific influenza risk assessments and propose potential sex-specific biological pathways leading to an increased risk for influenza in certain populations.

### Table 1. Selective Interaction Between Androgens, Estrogens, and Progestins With Cell Subsets of the Innate or Adaptive Immune System

<table>
<thead>
<tr>
<th>Hormone**</th>
<th>Effect on Immune Cell Subset†</th>
<th>Consequence During Influenza Infection‡</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Androgens</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testosterone (nmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women: 0.2–2.8</td>
<td>Inhibition of T-cell activation, dampening of Th1 differentiation, increased T-cell apoptosis</td>
<td>Potentially harmful due to impaired cytokine production and viral clearance</td>
</tr>
<tr>
<td>Men: 10–35</td>
<td>Inhibition of B-cell development and antibody production</td>
<td>Potentially harmful due to decrease of virus-neutralizing antibodies</td>
</tr>
<tr>
<td>Decreased TLR4 expression on innate immune cells</td>
<td>Potentially harmful due to poor induction of T-cell responses</td>
<td></td>
</tr>
<tr>
<td><strong>Estrogens</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estradiol (pmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women: 110–440 (follicular phase)</td>
<td>Activation of B cells and improved antibody response</td>
<td>Potentially beneficial due to increase of virus-neutralizing antibodies</td>
</tr>
<tr>
<td>330–1,200 (ovulation)</td>
<td>Promotion of T-cell lymphopoiesis</td>
<td>Potentially beneficial due to increased Th1 cytokine production</td>
</tr>
<tr>
<td>220–680 (luteal phase)</td>
<td>Increased number of recent thymic cell emigrants and associated greater naive T-cell pool</td>
<td>Potentially beneficial due to facilitated T-cell response</td>
</tr>
<tr>
<td>Up to 1800 (first trimester)</td>
<td>Increased frequency of regulatory T cells</td>
<td>Potentially beneficial due to maintenance of tissue hemostasis</td>
</tr>
<tr>
<td>Up to 75,000 (second trimester)</td>
<td>Th1 skew, production of IFN-γ, IL-18, IL-6, TNF</td>
<td>Potentially beneficial for viral clearance</td>
</tr>
<tr>
<td>Up to 136,000 (third trimester)</td>
<td>Facilitated recruitment of inflammatory cell via upregulation of chemokine receptor</td>
<td>Potentially beneficial for T-cell migration to lung and viral clearance</td>
</tr>
<tr>
<td>Men: 44–124</td>
<td>Anti-inflammatory actions (high levels, ie, during pregnancy)</td>
<td>Potentially harmful due to reduced viral clearance</td>
</tr>
<tr>
<td></td>
<td>Decreased production of B-cell progenitors (high levels, ie, during pregnancy)</td>
<td>Potentially harmful due to poor production of virus-neutralizing antibodies</td>
</tr>
<tr>
<td><strong>Progestogens</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Progesterone (nmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women: &lt;0.32 (follicular phase)</td>
<td>Enhanced chemotaxis of neutrophils</td>
<td>Potentially beneficial for amplification of inflammatory response</td>
</tr>
<tr>
<td>3.2–6.4 (ovulation)</td>
<td>Increased NK cell apoptosis</td>
<td>Potentially harmful due to poor DC activation and poor epithelial repair</td>
</tr>
<tr>
<td>15.9–38.3 (luteal phase)</td>
<td>Inhibition of Th1 bias</td>
<td>Potentially harmful due to reduced viral clearance</td>
</tr>
<tr>
<td>Up to 95 (first trimester)</td>
<td>Inhibition of TLR4-mediated innate immune response in macrophages</td>
<td>Potentially harmful due to insufficient induction of T-cell responses</td>
</tr>
<tr>
<td>Up to 210 (second trimester)</td>
<td>Suppression of DC maturation</td>
<td>Potentially harmful due to insufficient induction of T-cell responses</td>
</tr>
<tr>
<td>Up to 560 (third trimester)</td>
<td>Inhibition of TLR9-induced IFN-α production by pDCs</td>
<td>Potentially harmful due to poor induction of T-cell responses</td>
</tr>
<tr>
<td>Men: 0.9–3.8</td>
<td>Epigenetic silencing of chemokine recruitment (high levels, ie, during pregnancy)</td>
<td>Potentially harmful due to impaired T-cell migration to lung and viral clearance</td>
</tr>
</tbody>
</table>

Abbreviations: DC dendritic cell; IFN, interferon; IL, interleukin; NK, natural killer; pDC, plasmacytoid dendritic cell; Th1, T helper cell type 1; TLR, Toll-like receptor; TNF, tumor necrosis factor.

* Hormone levels are physiologically modulated by age in both sexes, as well as the menstrual cycle in women. The hormone values are given for serum levels in adult men and in adult, premenopausal, nonpregnant women.

† Described effects are compiled from observations in different species, cell subsets from different organs, from data in distinct models of autoimmunity or infection, or from in vitro experiments.

‡ The role of certain cell subsets may differ between the initiation, resolution, or restoration phase of the influenza infection, which should be taken into consideration and provides the rationale for using terms such as “potentially.” Page and reference constraints do not permit us to provide a fully referenced table including all original data sources. The interested reader is referred to the key reviews [2, 3, 7–10].
IMPACT OF SEX ON INFLUENZA MORBIDITY AND MORTALITY DURING SEASONAL INFLUENZA

Seasonal influenza epidemics occur yearly during autumn and winter and are caused by influenza A viruses circulating during the corresponding season. Worldwide, these epidemics result in approximately 3–5 million cases of severe illness, and about 250 000–500 000 deaths every year. The limited number of studies available to date reporting on patients’ sex indicate a male preponderance, albeit subtle, for influenza-like illnesses or confirmed influenza infections, for example, as shown across all age groups in Spain during the influenza seasons from 2002–2003 to 2005–2006 [14]. Using hospitalization rates as an indicator for influenza severity, studies from Denmark and Canada confirm a higher influenza risk for males compared to females across all age groups during the H1N1 and H3N2 influenza virus outbreaks between 1995–1999 and 1992–2001 [15, 16]. In Canada, retrospective analysis of hospitalization rates due to pneumonia and influenza between 1992 to 2001 and patients’ age reveal a particularly high risk for boys (0–14 years) and elderly men (>65 years) [15]. Similarly, higher hospitalization rates for young boys (<5 years) are reported in Denmark, whereas opposite findings are conveyed for women [16]. Data from Switzerland partly support the findings from Denmark and Canada, as an influenza-related death rate was reported for elderly men (>60 years) during the 1969–1999 seasons [17]. However, since the 2009 influenza pandemic (which is discussed in detail below), the awareness on the potential impact of sex on influenza disease outcome has increased and additional insights on sex-specific influenza risks can be anticipated in the future. This is already becoming evident in studies on the 2010–2011 influenza season. During this season, the 2009 pandemic H1N1 strains (2009 pH1N1) have become the dominant strains in Europe; the H3N2 subtype has been predominant in the United States, although 2009 pH1N1 strains also circulate. Overall, males are reported at higher risk for influenza in 2010–2011, with a male to female ratio of 1.3 in Europe, whereby younger age groups were substantially affected [18, 19]. Surprisingly, epidemiologic data on sex differences in countries with high populations such as the United States or China are not yet available on this season [19].

In 2011–2012, the second influenza season after the 2009 pandemic, H3N2 influenza virus has prevailed over the 2009 pH1N1 strains in Europe as well as in the United States. Among all confirmed influenza virus cases in Europe (n = 1269), the youngest and, to a greater extent, the elderly have been predominantly affected, along with a slightly higher risk for females (male to female ratio, 1.2:1) [20]. No sex-specific data are available yet from non-European countries [21].

In summary, it would be premature to postulate a sex-specific risk for seasonal influenza, due to the unavailability of sufficient epidemiologic data on past seasons. Hence, in addition to virus characterization in upcoming influenza seasons, surveillance studies should include data on potential sex-specific disease risks similar to the assessment of risk factors such as age or medical conditions.

IMPACT OF SEX ON INFLUENZA MORBIDITY AND MORTALITY IN ZOONOTIC INFLUENZA

Occasionally, influenza viruses may directly transmit from animal species to humans, including the avian influenza H5N1 and H7N9 viruses. Although human-to-human transmission is generally absent, avian influenza virus infections can lead to high case fatality rates in humans. To date, a total of 637 human cases with confirmed H5N1 influenza virus infections have been reported since 2003, with highest incidences in Indonesia, Egypt, and Vietnam. Available data indicate that females might be potentially more prone to H5N1 infections than males, and children and adolescents seem to be at greatest risk [22]. However, sex-specific risk assessment may be skewed in certain countries including Indonesia, where the population is composed of twice as many females as males [23]. On the other hand, no sex-specific risk for H5N1 infection could be detected in Vietnam [24], where a surplus in males is only a very recent phenomenon and hence might have affected risk assessments in children.

A very recent outbreak of human infections with the avian H7N9 subtype was reported in China, and pilot data indicate that males are more prone to severe disease outcome than females [25]. However, sex aspects need to be taken into account in this context, as men are more likely to work with potentially infected poultry. Contact with poultry may also not be the sole risk factor for severe H7N9 influenza, as elderly, hence, likely retired individuals are also severely affected [26].

IMPACT OF SEX ON INFLUENZA MORBIDITY AND MORTALITY DURING PANDEMIC INFLUENZA

Irregularly occurring pandemic influenza seasons affect societies worldwide. The most serious pandemic in recorded history was the “Spanish flu,” which led to the deaths of approximately 50 million people in 1918/19. Reexamination of statistical data available on influenza-related fatalities in the United States and other countries revealed that the 1918 pandemic had a significant sex bias, as death rates were higher among males than females [27]. However, the high mortality rate has often been attributed to coinfections with other pathogens, such as tuberculosis, which was highly prevalent during 1918 and disproportionately higher in males [28]. Coinfections with other potential pathogens that can be enhanced by influenza infection include streptococci, pneumococci, or human immunodeficiency virus.
Thus, it is difficult to assess female–male differences when data are not stratified for coinfections with other pathogens after primary influenza. Another study also reported increased death rates for men during the 1918 influenza pandemic in Australia, which has been suggested to result from a gender-specific social behavior, including workforce participation rates and attendance in crowded places such as bars or sporting events [29]. Moreover, the coincidence of the 1918 pandemic with World War I may have also contributed to an enhanced virus exposure for men.

Between 1956 and 1958, the “Asian flu” pandemic resulted in death toll ranging from 1 to 4 million people. Serfling and colleagues report that morbidity and mortality during this pandemic was increased among adolescent girls, which has been proposed to result from the many opportunities for contacts among teenagers at that time and associated potential for viral exposition [11]. Although this seems a feasible explanation, one wonders if such social behavior–associated risk could also be applicable for male teenagers during the 1950s.

The first pandemic of this century has been caused by the emergence of a novel H1N1 strain in 2009. Worldwide, 201 200 respiratory deaths with an additional 83 300 cardiovascular deaths have been associated with 2009 pH1N1 infections. Eighty percent of the respiratory and cardiovascular deaths affected people <65 years of age and 51% occurred in Southeast Asia and Africa [30]. In contrast to the 1918 pandemic, female–male differences were less evident during the 2009 pandemic. In a very recent study on data collected from 70 000 cases in 19 countries, a high risk for hospitalization is reported in patients <5 years and between 5 and 14 years of age, where the elderly show highest mortality rates. No significant sex-specific risk has been reported across all age groups and countries with regard to hospitalization rate or mortality [31].

Taken together, sex-specific risk assessment for influenza infections during pandemics is, similar to seasonal epidemics, difficult to ascertain, largely due to limited availability of sex-specific surveillance data, the sex-induced bias of influenza infections, and the difficulties to distinguish influenza virus infection from influenza-like illnesses or acute respiratory infection. Although these conclusions are rather unsatisfactory due to their vagueness, one undisputable observation recurrently observed during influenza infections over the last century is the significantly increased risk for influenza among pregnant women, particularly during the pandemics.

**PANDEMIC INFLUENZA AND PREGNANCY**

During the 2009 pandemic, pregnant women were instantly recognized to be at higher risk for influenza-related morbidity and mortality than the general population. The risk for influenza appears to increase with increasing gestational age [32], whereby a large number of epidemiologic studies fail to provide details on gestational age at time of infection. Also, some women may have been unaware of their pregnancy very early during gestation, which may bias trimester-dependent risk assessments. Pregnant women were approximately 7 times more likely to be hospitalized and 2 times more likely to die from influenza than age-matched nonpregnant women [31, 33]. In the United States, pregnant women accounted for 5% of all deaths, although they only represent 1% of the general population [34]. Similarly, pregnant women have also been identified to be more vulnerable to severe influenza than the general population during the 1957–1958 Asian flu pandemic [35, 36], mirrored by high maternal mortality rates and as increased miscarriages, premature birth, and neonatal death [36]. These observations from the past pandemics have now resulted in a revised recommendation by the World Health Organization Strategic Group of Experts, declaring pregnant women to be the highest priority group in seasonal influenza vaccination programs [37]. Yet, underlying pathways rendering pregnant women at high risk for influenza and associated morbidity and mortality for mother and (unborn) child still remain to be identified.

**IMMUNE ADAPTATION DURING PREGNANCY AND POTENTIAL IMPACT ON SEVERE INFLUENZA OUTCOME**

During pregnancy, progesterone and estradiol levels significantly increase. It is noteworthy that an increased production of other hormones such as pituitary-derived prolactin or placenta-derived human chorionic gonadotropin and glucocorticoids also occurs during pregnancy. This physiological hormonal adaptation contributes to the maintenance of pregnancy in mammals, as sex hormones engage in a cross-talk with immune cells and contribute to a precondition of tolerogenic immune pathways. Such immune tolerance is required to suppress the rejection of paternally derived semiallogenic antigens expressed on fetal tissues [7]. Immune pathways involved in such fetal immune tolerance are displayed in Figure 1 and summarized in the figure legend. Estrogens have been proposed to enhance the immunity in females, but the high risk for influenza during pregnancy clearly rejects the notion that pregnancy-induced high levels of estrogens may protect from influenza. This may be due to the multifaceted, dose-dependent function of estrogens on the immune system, which can trigger an anti-inflammatory response if levels are high (Table 1). Moreover, one might argue that progesterone may further aggravate the severely impaired immunity to clear influenza during pregnancy by perpetuating the skew toward anti-inflammation. Insights from influenza risk susceptibility throughout the menstrual cycle (although levels of both hormones are significantly lower compared to pregnancy) may be helpful to dissect the differential effect of estrogens to progesterone. Surprisingly, such
Figure 1. Contradictory demands for the immune system to adapt to pregnancy and to simultaneously mount an immune response to clear the influenza virus. Left panel: During pregnancy, the maternal immune system adapts to pregnancy to mount immune tolerance toward the semiallogeneic fetus and ensure placental vascularization and fetal growth. Such adaptations include the abundance of regulatory rather than cytotoxic natural killer (NK) cells at the fetomaternal interface. Uterine NK cells become licensed during pregnancy, i.e., upon interaction with human leukocyte antigens expressed on extravillous trophoblast cells (EVTs). Moreover, dendritic cells (DCs) present at the fetomaternal interface are confined to a tolerogenic phenotype (tDC), which reduces the priming of antifetal effector T (Teff) cells and promotes the expansion of CD4+ regulatory T cells. Protection of fetal immune tolerance is further achieved by the targeted deletion of fetal antigen–reactive T cells (not displayed). Moreover, the B-cell response is also altered during pregnancy, as reflected by a reduction of circulating B cells in normally progressing human pregnancies. The latter has been proposed to result from the estrogen-mediated suppression of B-cell lymphopoiesis [38]. Additionally, recent observations in mice support that the placenta evades effector T-cell–mediated rejection by sex hormone–induced epigenetically silenced production of chemokine receptor ligands in the decidua. Here, via histone modification (HM), the migration of antifetal Teff cells to the decidua may be averted [39]. Many of these maternal immune adaptations to pregnancy are triggered by the surge of pregnancy hormones [4, 8]. Right panel: Upon H1N1 influenza infection, antiviral immunity has to be mounted, and the innate immune response in the lung is initiated upon virus detection by pattern recognition receptors (not shown). Innate immune cells including NK cells and DCs are recruited from the circulation to the lung airways and lung parenchyma. NK cells infiltrate the lungs early during influenza virus infection and functional NK cell activation can be triggered by the interaction between viral hemagglutinin on infected cells including epithelial or dendritic cells and cytotoxicity receptors on the NK cell surface. In turn, NK cells become activated (licensed) and release perforin, granzymes (Gr), and antiviral mediators such as interferon gamma (IFN-γ) and tumor necrosis factor (TNF). Moreover, the migration of activated, virus antigen-presenting cells (APC) to the lung-draining lymph node is another pivotal aspect of the early immune response to the infection. Upon migration to the lung-draining lymph node, DCs interact with naive T cells and initiate the adaptive immune response, i.e., the generation of virus-specific Teff cells and B cells. Chemokine receptor ligands facilitate the recruitment of CD4+ and CD8+ Teff cells to the lung airways and parenchyma. Virus clearance is achieved by lysis of infected epithelial cells and virus-specific B-cell–derived antibodies such as immunoglobulin M (IgM) and immunoglobulin G (IgG).
insights are sparse, as only one study is available to date on such topic. Although the sample size is rather low, this study reports an increased risk for influenza infection commencing at ovulation and during the luteal phase of the cycle. Because both progesterone and estradiol are increased during the luteal phase, it is tempting to speculate that estrogens enhance immunity only if unopposed by progesterone, as present during the follicular phase.

To provide a rationale for the increased risk for influenza during pregnancy, one can postulate that high risk is a collateral effect of the maternal endocrine and immune adaptation to pregnancy. This adaptation may create a contradictory demand for the immune system. It can be proposed that during pregnancy, distinct cell subsets are “locked” into a phenotype, that is, by sex/pregnancy hormones. In turn, these cell subsets—or respective progenitor cells—fail to acquire a phenotype and to mount the immune response required to clear the influenza virus infection (Figure 1). A description of markers and mediators of the immune response required to clear the in-

Conclusions

Given that our understanding of a sex-specific effect involved in influenza infection is still rather limited and findings from epidemiologic studies reveal ambiguity, we propose that the following gaps be addressed in future studies: (1) to dissect surveillance data for sex and possibly menstrual cycle stage in addition to the focus on virus characteristics, age groups, stratification of data for coinfection, and geographical settings; (2) to firmly include a focus on influenza susceptibility during pregnancy in surveillance studies. In this regard, the growing availability of basic science models is expected to yield to the identification of pathways causal for the greater risk for influenza during pregnancy. Such studies will be advanced if research endeavors are carried out in interdisciplinary teams, preferably including the areas of virology, reproductive biology, and immunology. Collateral effects associated with maternal immune adaptation to pregnancy, which may render the immune system inept to deal with the influenza virus infection, can then be identified in the near future. Once identified, such understanding may not immediately be translated into therapeutic interventions due to the required exclusion of potential teratogenic effects. However, the growing knowledge of why pregnant women are at greater risk for influenza can be anticipated to increase communities’ alertness and improve the poor vaccination compliance rate among women during their reproductive years.

Notes

Financial support. This work was supported by grants provided by the Foundation for Research and Science Hamburg, the Werner Otto Foundation, and the German Research Foundation to P. C. A. and the
Emmy-Noether Research Programme from the German Research Foundation to G. G.

Potential conflicts of interest. Both authors: No reported conflicts.
Both authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References