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The effect of sex on responses to influenza vaccines

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Corresponding Author:	Lucy Rose Denly, BA University of Oxford OXFORD, Select County UNITED KINGDOM
First Author:	Lucy Rose Denly, BA
Order of Authors:	Lucy Rose Denly, BA
Manuscript Region of Origin:	UNITED KINGDOM
Abstract:	<p>The poor uptake and limited effectiveness of seasonal influenza vaccines means that influenza continues to create a significant burden of disease. It has been hypothesised that sex differences are present in responses to seasonal influenza vaccines, and that these differences may contribute to this poor vaccine success. This has led to the suggestion that vaccines should be tailored to an individual's biological sex. However, studies in this field are often low quality. Comprehensive analysis of the available literature reveals that there is insufficient evidence to support sex differences in vaccine immunogenicity, effectiveness, or efficacy. Nonetheless, differences in vaccine safety are consistently observed, with females reporting adverse events following immunization more frequently than males. Bias introduced by gender differences in passive reporting of adverse effects may underlie this phenomenon. Highly-controlled studies are required in future before any conclusions can be made about potential sex differences in response to seasonal influenza vaccines.</p>

1 **The effect of sex on responses to influenza vaccines**

2 Lucy Denly^{a*}

3 ^a*University of Oxford, Oxford, UK*

4 *lucy.denly@sjc.ox.ac.uk

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The effect of sex on responses to influenza vaccines

The poor uptake and limited effectiveness of seasonal influenza vaccines means that influenza continues to create a significant burden of disease. It has been hypothesised that sex differences are present in responses to seasonal influenza vaccines, and that these differences may contribute to this poor vaccine success. This has led to the suggestion that vaccines should be tailored to an individual's biological sex. However, studies in this field are often low quality. Comprehensive analysis of the available literature reveals that there is insufficient evidence to support sex differences in vaccine immunogenicity, effectiveness, or efficacy. Nonetheless, differences in vaccine safety are consistently observed, with females reporting adverse events following immunization more frequently than males. Bias introduced by gender differences in passive reporting of adverse effects may underlie this phenomenon. Highly-controlled studies are required in future before any conclusions can be made about potential sex differences in response to seasonal influenza vaccines.

Keywords: seasonal influenza vaccine; sex differences; vaccine safety; vaccine efficacy; vaccine effectiveness; vaccine immunogenicity

Subject classification codes: influenza ; vaccinology

Introduction

Despite biannual re-evaluation and reformulation, seasonal influenza vaccines remain unsuccessful in preventing disease, with influenza accounting for between 291 243 and 645 832 deaths annually worldwide¹. This is a result of both poor uptake and low vaccine effectiveness. Estimates of influenza vaccine effectiveness range from 10% to 60%², in contrast to vaccines for other common viral diseases. For instance, the measles vaccine is effective in 97%³. Mismatch due to antigenic drift is often the main barrier to vaccine success, which reformulation often fails to circumvent. This is highlighted in Figure 1. Different types of vaccines have been trialled to improve outcomes. Age is an important determinant for their success; this has been useful in informing vaccine recommendations, as shown in Table 1. For example, the quadrivalent influenza vaccine (QIV) was recommended in children and young adults since it was more protective against influenza B subtypes than the trivalent influenza vaccine (TIV)⁴. However, no benefit of the QIV was seen in older adults, who are less susceptible to influenza B infection, thus the TIV is still recommended for this age group⁵. Age-specific differences have also been reflected in vaccine design. For example, an adjuvanted TIV was introduced in the 2018-19 season for those over 65 following observations of poor effectiveness of the standard TIV in this age group. This has resulted in better prevention of flu⁶.

While age-specific differences in outcomes have been accounted for in vaccine strategy, sex differences have been largely ignored. Sexual dimorphism has been observed following immunization against numerous pathogens⁹. The mechanisms underlying these differences are not well understood, and have been speculated elsewhere^{10,11}. Recently, certain research groups have hypothesised that responses to seasonal influenza vaccination differ between males and females^{9,12,13}. This has led to

the suggestion that vaccine design should be tailored to sex. If true, these differences could contribute to the poor effectiveness of seasonal influenza vaccination. This mini-review aims to explore whether sexual dimorphism exists in seasonal influenza vaccination, through focusing on the available literature from model animal and human studies. Possible implications of any differences will be discussed, including whether sex-specific vaccines should be introduced.

Sex differences in immunogenicity are present in mouse models of influenza vaccination

Although the presence of sex differences in immune responses is widely acknowledged¹¹, preclinical vaccine studies rarely report the sex of animals used, let alone investigate differences in responses between sexes. Biannual reformulations of seasonal vaccines do not require preclinical testing for approval. Consequently, there are few preclinical studies of seasonal influenza vaccination, and even fewer investigating sex differences. The studies that have been performed have all used mice, which are the most widely used model animals in pre-clinical vaccine studies¹⁴. A number of reagents allowing measurement of immunogenicity can be used in mice¹⁴, including functional antibody assays (viral microneutralization (MN) and hemagglutinin inhibition (HAI) assays) which are commonly used to assess vaccine-mediated protection against influenza in humans. Titres of IgG are also measured due to its important role in vaccine-mediated protection¹⁶. These measures of immunogenicity are relative correlates of protection in humans¹⁵.

Studies investigating vaccine immunogenicity have shown that female mice develop greater neutralizing antibody and IgG titres against immunized inactivated H1N1^{17–21} and H3N2^{17–20}. For example, in a study by Živković et al (2015), total IgG, HAI and MN titres against H1N1 in male mice were on average half of that of females

at both 3- and 6-weeks post-immunization. However, this study only found differences between males and females for total IgG titres in response to H3N2 or B influenza strains, with no significant differences found in MN or HAI titres¹⁷. There is no clear consensus on sex differences in antibody quality. IgG avidity was shown to be greater in females than males in one study²¹, while another study showed no difference¹⁹. Recent studies have implied IgG avidity changes with age, yet data here are conflicting too. One study shows no sex difference in young mice, with strain-specific IgG showing around 40% avidity, but a greater avidity of 60% or more in aged females with no such increase seen in aged male mice²⁰. Another study shows the opposite effect, with young female mice showing an avidity index of 0.3 compared to 0.15 in young males, while aged mice of both sexes show an index of around 0.15²². This study investigated responses to an inactivated 2009 H1N1 influenza vaccine only. Further studies are required to understand why these data are conflicting.

Sex differences have also been observed in response to live attenuated influenza vaccines (LAIV). Neutralizing and total antibody responses to H1N1 or H3N2 influenza strains were higher in female mice following immunization than in age-matched male mice²³. These differences were not consistent across all time points measured. For example, females showed almost double the total anti-H1N1 IgG of males at 14- and 21-days post-immunization but no difference was observed at 28 days. A similar difference was seen between males and females for total anti-H3N2 IgG at 28 days but not at 14 or 21 days. No significant differences in morbidity or mortality were observed between females and males upon challenge with a homologous virus²³. Female mice showed slightly lower morbidity on challenge with a heterosubtypic strain of influenza (e.g. H3N2 challenge following initial vaccination with H1N1)²³, however cross-protection has rarely been observed in human studies so it is unlikely that this sex

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102 difference is present in humans. This highlights one aspect in which mouse models
103 poorly replicate human influenza pathophysiology.

104 A further factor which makes these immunogenicity studies poorly
105 representative of human vaccination is the use of anaesthesia during vaccination^{21,23}.
106 Anaesthesia alters the immune response to influenza in mice, with infection under
107 anaesthesia resulting in a viral pneumonia which is unrelated to upper airway influenza
108 infection observed in awake mice and humans²⁴. It is likely that vaccination under
109 anaesthesia also alters immune responses. This makes these studies unsuitable for
110 understanding sex differences that may be present in human immune responses to
111 vaccination.

112 The use of inbred commercial mice strains in these studies may also interfere
113 with our understanding of human sex differences following influenza vaccination^{19–21,23}.
114 X chromosome inactivation has been shown to have no effect on immune responses in
115 these inbred strains, unlike in humans and outbred animals. This implies sex differences
116 observed in these mice models may be very different to sex differences present in
117 humans. Using outbred mice may be more suitable to modelling human sex-differences
118 in vaccine immune responses^{17,18}.

119 Few studies have linked immunogenicity with protection by challenging mice
120 with influenza following vaccination. Two studies have associated greater antibody
121 responses in vaccinated females to faster viral clearance and lower morbidity following
122 challenge with a H1N1 drift variant^{21,22}. In a study by Fink et al (2018) female mice had
123 twice the IgG titres and triple the neutralizing antibody titres of males²¹. These female
124 mice exhibited an average 3% loss in body mass compared to 8% loss of body mass in
125 males²¹. While the body mass of females had returned to normal 4 days post-challenge,
126 male body mass was still lower than normal 14 days following challenge²¹. Antibody

127 titres from vaccinated females were also better at protecting naive mice than antibodies
128 from vaccinated males²¹. This increased protection was seen in young mice only,
129 highlighting that potential sex differences may be dependent on age. More challenge
130 studies such as these are required in future to determine whether sex differences in
131 immunogenicity correspond to differences in protection. However, a major issue is
132 encountered when challenging vaccinated mice with influenza strains. Most human
133 influenza strains do not replicate efficiently in mice without prior virus adaptation
134 through serial passages in the lungs. Some well adapted strains, such as the Influenza
135 A/Puerto Rico/8/34 H1N1 used by Lorenzo et al. (2011)²³, have undergone numerous
136 mutations altering their antigenicity and replication kinetics in order to cause disease in
137 mice²⁴. Consequently, challenge of vaccinated mice with these strains poorly replicates
138 human challenge with circulating influenza strains. It is thus unlikely that the
139 differences in vaccine-induced protection observed between sexes in this study reflects
140 human differences.

141 Selection of animal models that accurately reflect human immune responses
142 remains one of the major challenges facing influenza vaccination research¹⁴. Thus far
143 only mice have been used to investigate sex differences. These studies have shown that
144 females generate greater humoral responses to influenza vaccines than males. However,
145 dissimilarities in influenza vaccination between mouse models and humans means that
146 it is impossible to extrapolate these sex differences onto human populations. Directly
147 investigating responses in humans to influenza vaccination will most accurately reveal
148 any sex differences. Nonetheless, pre-clinical studies are much easier to conduct under
149 highly controlled conditions than human trials, and so are less effected by confounding
150 factors. There is an immediate need for more preclinical vaccine studies to be designed
151 with equal numbers of males and females, and with the aim of investigating sex-

dependant differences in outcome measures. Ferrets should be used to continue preclinical investigation into the sex differences observed in mice, as they model human influenza pathophysiology more representatively²⁴.

Clear sex differences are not present in human responses to influenza vaccination

Immunogenicity studies

As is the case for preclinical studies, clinical trials are not required for seasonal influenza vaccine updates. Furthermore, clinical trials rarely evaluate sex differences, so few human studies have investigated the sex differences in seasonal influenza vaccine outcomes. The studies that have been done mainly assess vaccine immunogenicity. These usually measure humoral immune response to vaccination using a hemagglutinin inhibition assay (HAI), in which a 1:40 antibody titre corresponds to strain-specific protection following immunisation with an inactivated influenza vaccine. There is no suitable correlate of protection for the LAIV, so few studies investigating LAIV immunogenicity have been carried out. A few studies observed antibody responses to be greater in females than males following immunization^{25–29}. For example, post-hoc analyses carried out by Falsey et al (2009) in a randomised controlled trial of the TIV found females to have greater HAI geometric mean titres²⁷. This difference was most pronounced for H3N2-specific titres, which had a mean value of 382.8 in females compared to 280.4 in males following immunization with a standard TIV dose²⁷. These findings have led to some research groups hypothesising immunogenicity to be greater in females following vaccination than males^{21,30–32}. However, observed differences are rarely clear cut. For example, Cook et al. reported a higher immunogenicity in females than males for all influenza strains following intramuscular TIV immunization, but observed no differences at all following subcutaneous TIV immunization²⁶. Furthermore, the study by Falsey et al (2009) found no significant sex differences for

177 other influenza strains, with geometric mean titres for males and females registering at
178 52.1 and 52.5 respectively for B-specific titres²⁷.

179 Numerous studies reveal either a higher immunogenicity in males^{31,32}, or no sex
180 differences in immunogenicity following influenza vaccination^{26,33–43}. The latter by far
181 outnumber those studies showing the presence of sex differences. A systematic review
182 by Tadount et al. (2019) highlighted that of the published studies investigating sex
183 differences in influenza vaccine immunogenicity, the majority found no sex
184 differences⁴⁴. Only studies investigating adult participants were included, so whether
185 this is the case for children remains unknown. Meta-analyses using individual
186 participant data from the original trial data sets would be a more reliable approach to
187 investigating sex differences than a systematic review. However, a meta-analysis could
188 not be conducted due to the heterogeneity of study populations and the lack of precision
189 in reported measures. Nonetheless, this review implies that this hypothesis is
190 unsupported by the available evidence.

191 *Efficacy and Effectiveness Studies*

192 Immunogenicity does not provide a measure of vaccine success; for this, disease
193 prevention among vaccinated populations must be assessed. The gold standard
194 measurement for disease prevention is vaccine efficacy, measured in randomised
195 control trials (RCTs) and defined as the percentage reduction in disease incidence in a
196 vaccinated group compared to an unvaccinated group under optimal conditions³⁰. These
197 studies usually use laboratory diagnostic tests to assess influenza status. Nonetheless,
198 RCTs investigating laboratory-confirmed infection are affected by confounding factors,
199 such as the sensitivity and specificity of the diagnostic tests⁴⁵.

200 No RCTs have been carried out with the primary aim of investigating sex
201 differences in influenza vaccine efficacy. A few studies separate male and females,

allowing efficacy values for each sex to be estimated. Rarely were sex differences in efficacy evaluated in these studies, as it is poor practice to assess subgroup comparisons not prespecified in the trial protocol⁴⁶. A single efficacy study stratified by sex met eligibility criteria to be included by Tadount et al (2019) in their systematic review⁴⁴. This phase III RCT found a significantly higher TIV efficacy in healthy young males compared to age-matched females³². This result is likely to be affected by interference of confounding factors such as vaccination history. The female group of this study had higher previous immunization rates, thus increased exposure to viral antigens. This could underlie the weaker immune response observed when compared to males⁴⁷.

Carrying out placebo-controlled trials in populations for which vaccination is recommended is unethical⁴⁸, and so RCTs are often unsuitable. Instead, observational studies are often used to estimate vaccine effectiveness, defined as the ability of vaccines to prevent outcomes of interest in the “realworld”⁴⁹. Effectiveness studies adjust for confounding factors. Sex is often considered a confounder⁵⁰, and its effect eliminated through multivariate analysis. Some studies use stratification instead to provide an effect measure for each sex stratum. This enables comparison of effectiveness between sexes. Unfortunately, influenza vaccine data is rarely stratified by sex. Sex-stratified studies of influenza vaccine effectiveness are reviewed by Tadount et al (2019)⁴⁴. Most effectiveness studies are not designed to investigate sex differences, and sex stratified effectiveness values had to be adjusted for age, health status and vaccination history, in order for the risk of bias to be deemed low enough for inclusion in the review⁴⁴. Studies with a high risk of bias were excluded⁴⁴.

Many effectiveness studies use clinically relevant health outcomes rather than laboratory-based outcomes. All-cause mortality from influenza-like illness is often used as an outcome. Some studies have used this outcome to imply that influenza vaccine

effectiveness in greater in females than in males^{51–53}. However, the use of such outcomes compromises the reliability of these findings, as the criteria used to define influenza-like illnesses lacks specificity for influenza diagnosis⁵⁴. Test-negative designs minimise this issue by using laboratory-based diagnostic tests, as illustrated in Figure 2. Tadount et al. (2019) only considered studies using TNDs in their review⁴⁴. Two effectiveness estimates showed no differences between older males and females^{55,56}. A further four low-quality studies gave more crude estimates of effectiveness, and also showed no differences^{57–60}. While TNDs aim to minimise bias in these studies, gender differences in health-seeking behaviours may introduce selection biases that interfere with sex-specific estimates⁴⁹. Evidence implies that men show delayed health-seeking behaviour and women do not⁶¹. Men with may be less likely to seek care for influenza-like illness than women when symptoms are mild. However, men with severe symptoms will seek help and be tested for influenza. These men are more ill and more likely to test positive for influenza. Therefore, while fewer men get tested for influenza, a higher proportion will test positive. This may affect sex-specific effectiveness estimates. The low quality of these studies meant that generation of pooled sex-stratified effectiveness estimates was not possible, and this data could not reliably be used to evaluate sex differences in vaccine effectiveness.

Safety studies

Vaccine safety is crucial and is strictly monitored prior to authorization. Currently licensed influenza vaccines display good safety profiles across the age groups in which they are used⁶². However, as influenza vaccines are administered to large proportions of the population, adverse effects following immunization (AEFI) may affect a significant number of individuals. AEFIs can be local (e.g. site injection pain/redness/swelling) or systemic (e.g. fever). Changes in vaccine formulations and

host or environmental factors may affect the prevalence or severity of AEFIs.

Consequently constant monitoring of safety is essential⁶³. This is achieved through the routine use of surveillance systems which rely on detection by health professionals or passive reporting by the recipient.

Unlike other vaccine responses, vaccine safety does appear to differ between sexes. Overall, 21 of the 35 investigated study groups included in the review by Tadount et al (2019) revealed females experienced AEFIs more frequently than males⁴⁴, such as in the study by Govaert et al (1994) in which females showed twice the rate of AEFIs (30%) than males (15%)⁴¹. This was most pronounced in studies into local reactions, with 9 of the 10 studies showing significantly higher rates in females. Again, there is a risk that gender differences in health-seeking behaviour may affect the data of these studies; men experiencing AEFIs may be less likely to report this to a physician than women, which could lead to underreporting in male AEFIs⁶¹. This bias may be more prevalent in local reactions than systemic reactions, as these are less severe. This may reflect why only 5 of 13 studies showed higher incidence of systemic reactions in females, with the other 8 studies finding no sex differences. While meta-analysis of this data was not possible due to heterogeneity of effect measures, there appears to be a definite trend towards females reporting more AEFIs than males. It is yet unclear whether this is because of this reporting bias. There is a need for collection of AEFI data through methods other than passive reporting to investigate whether this trend is a true biological phenomenon.

Conclusions and Future Perspectives

While animal studies and a few human studies support the hypothesis that influenza vaccine immunogenicity is greater in females, much of this data is unreliable.

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277 Mouse studies poorly model sex differences that may occur in humans, and there is a
278 need for more physiological models of human influenza vaccination need to be used in
279 future. Human studies reporting no sex differences vastly outnumber those that report
280 sex differences. Consequently, the suggestion that male-specific vaccines designed to
281 potentiate immunogenicity should be introduced is unsupported by the available
282 evidence¹².

283 Evaluation of effectiveness and efficacy studies into influenza vaccines does not
284 support the existence of sex differences in vaccination outcomes¹². There is a distinct
285 lack of high-quality research investigating sex differences, which makes this evaluation
286 difficult. Large-scale efficacy studies must be carried out in future, with the aim of
287 investigating sex differences. These should provide more reliable insights into whether
288 sex differences are present in vaccine-mediated disease prevention.

289 Females have been consistently observed to report AEFIs more frequently than
290 males in safety studies. This has been suggested to contribute to the increased vaccine
291 hesitancy observed among women^{10,64,65}. However willingness to report AEFIs may
292 differ between men and women, and this is likely to play a role in this observed
293 difference. Some have suggested vaccines with lower reactogenicity should be designed
294 for females to reduce AEFI frequency while retaining protective antibody responses¹².
295 This could be achieved through lowering the vaccine dosage. A large phase II trial
296 conducted by Engler et al (2008) demonstrated that the antibody response of females to
297 a half dose of TIV was equivalent to that of a full dose in males⁶⁶, implying that half the
298 usual TIV dose may be sufficient in females. However, there is so-far insufficient
299 evidence to support the proposal that AEFIs are indeed higher in females, or that sex-
300 specific vaccines should be designed given the logistical complexity in managing
301 different influenza vaccine for both different age and sex populations. It is crucial that

highly controlled trials are carried out investigating this trend for more frequently reported AEFIs in females. If this phenomenon is indeed biological, it may underly the increased vaccine hesitancy observed in females^{64,65}. By reducing the rate of these AEFIs in females, more females may choose to receive a seasonal influenza vaccine each year. thus a greater proportion of the population would be protected from the predicted circulating influenza strains. This could be crucial in reducing the number of seasonal influenza deaths in future.

This mini-review focuses on the available literature, and hopes to provide a balanced view of the present data. Nonetheless, the limitations of the literature mean biases may have impacted any conclusions drawn. Sex differences are rarely considered in any study and are never the main purpose of a clinical vaccine trial, therefore the published literature is full of reporting and publication bias. Re-analysis of the existing data, including those unpublished on sex differences, may help to reduce these biases in the literature. It is hoped that the increased availability of individual participant data in clinical trials in recent years will allow more meta-analyses of sex differences to be carried out in future, therefore providing high-quality data on sex differences which are currently not available.

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497 Table 1. NHS influenza vaccine recommendations for the 2019-2020 season⁸.

498

499 Figure 1. Antigenic mismatch decreases effectiveness of seasonal influenza vaccines⁷.

500 The prevalent circulating strains are selected 6 months before the beginning of the flu

501 season, to allow adequate time for vaccine production. These strains are grown in eggs.

502 Mutations may occur in the vaccine strains to increase their ability to grow in eggs (egg-

503 adaptation). Mutations may occur in the circulating strains between selection and

504 vaccine administration (genetic drift). Both types of mutations may lead to antigenic

505 mismatch, so vaccinated populations do not have protection against the influenza strains

506 circulating at the time. This leads to low vaccine effectiveness for that season.

507

508 Figure 2. Test-negative design studies. A form of observational study commonly used in

509 effectiveness studies of influenza vaccination to reduce disease misclassification and

510 confounding by health care-seeking behaviours. However, gender differences in health-

511 seeking behaviour may introduce selection bias.

512

Table 1

Age group	Recommended vaccine	Reason for recommendation
< 6 months	Not recommended	Too young to generate protective immunity
6 months- 2 years	Egg-grown quadrivalent inactivated influenza vaccine (QIV)	Protects against four influenza strains (H1N1, H3N2, and two B strains), providing better protection than trivalent IIV against the circulating flu B strains which are more likely to affect younger patient group.
2-17 years	Live attenuated influenza vaccine (LAIV)	Protects against four influenza strains (H1N1, H3N2, and two B strains), providing better protection than trivalent IIV against the circulating flu B strains which are more likely to affect younger patient group. Intranasal vaccine to reduce spread of flu virus in children. Contraindicated in many groups. Live vaccine induces broader immunity but increased reactogenicity.
18-64 years	Egg-grown quadrivalent inactivated influenza vaccine (QIV)	Protects against four influenza strains (H1N1, H3N2, and two B strains), providing better protection than TIV against the circulating flu B strains which more likely to affect younger patient groups.
	Cell-grown quadrivalent inactivated influenza vaccine (cQIV)	Protects against four influenza strains (H1N1, H3N2, and two B strains), providing better protection than TIV against the circulating flu B strains which more likely to affect younger patient groups.. May be an advantage to using IIVs which have adapted to growth in eggs, however insufficient evidence of superior protection
>65 years	Adjuvanted trivalent inactivated influenza vaccine (adjuvanted TIV)	Protects against three influenza strains (H1N1, H3N2, and one B strain).Adjuvant increases immunogenicity and vaccine effectiveness than standard inactivated vaccines, aimed to counter immunosenescence.
	High-dose trivalent inactivated influenza vaccine (TIV-HD)	Protects against three influenza strains (H1N1, H3N2, and one B strain). Contains four times the antigen dose of standard inactivated vaccines, aimed to enhance immune response to counter immunosenescence.

Figure 1

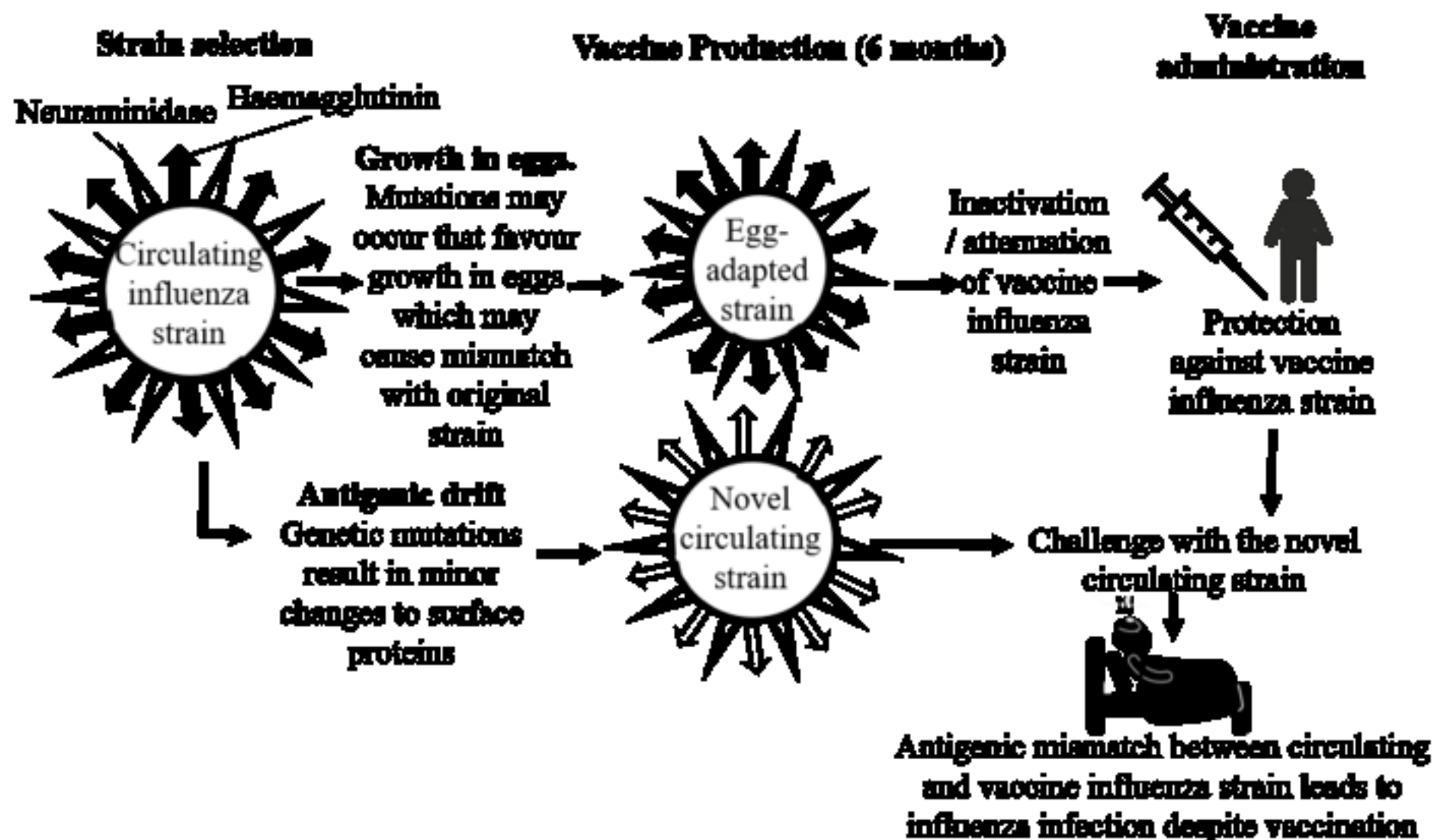


Figure 2

