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Developmental and reproductive safety of AZD1222 (ChAdOx1 nCoV-19) in mice

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**A B S T R A C T**

AZD1222 (ChAdOx1 nCoV-19) is a COVID-19 vaccine that is not yet licensed for use during pregnancy. To support the inclusion of pregnant and breastfeeding people in AZD1222 clinical studies, a non-clinical developmental and reproductive toxicity study was performed to evaluate its effects on fertility and reproductive processes of female CD-1 mice during the embryofetal development phase, and postnatal outcomes during the littering phase. Immunogenicity assessments were also made in dams, fetuses, and pups. There were no vaccine-related unscheduled deaths throughout the study. Furthermore, there were no vaccine-related effects on female reproduction, fetal or pup survival, fetal external, visceral, or skeletal findings, pup physical development, and no abnormal gross pathology findings in pups or dams. Antibody responses raised in dams were maintained throughout gestation and postnatal periods, and seroconversion in fetuses and pups indicate placental and lactational transfer of immunoglobulins. Together with clinical data from non-pregnant people, these results support the inclusion of pregnant and breastfeeding people in AZD1222 clinical studies.

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of coronavirus infectious disease-2019 (COVID-19) [1]. Studies of previous human coronavirus (HuCoV) outbreaks, including the 2002–2004 SARS outbreak and the 2012–2013 Middle East respiratory syndrome outbreak [2,3], suggest that pregnant females and their fetuses may be particularly susceptible to poor outcomes following HuCoV infection [4,5] because of physiologic changes in the immune and cardiopulmonary systems during pregnancy [6].

To date, in the United States alone, there have been almost 100,000 recorded cases of pregnant people with COVID-19 [7]. Limited data exist on the clinical features of this disease during pregnancy, and there are few large cohort studies providing data on maternal, fetal, perinatal, and neonatal outcomes following SARS-CoV-2 infection. However, clinical trials to evaluate the relationship between COVID-19 in pregnancy and adverse perinatal outcomes and to determine, for example, the rate of SARS-CoV-2 infection in people presenting with miscarriage and stillbirth, and the risk and characteristics of vertical transmission, are ongoing [8–10]. Currently there is no evidence to suggest that SARS-CoV-2 causes birth defects, or that maternal COVID-19 is associated with miscarriage, and findings in a small number of studies do not indicate a higher risk of stillbirth or increased death rates in neonates of mothers with suspected or confirmed COVID-19 compared with uninfected mothers [11]. However, pregnant people with COVID-19 are at increased risk of requiring admission to an intensive care unit compared with non-pregnant people of reproductive age, and may be at increased risk for severe respiratory complications and delivering preterm compared with uninfected pregnant people [12,13]. While most babies born to people with COVID-19 are healthy, vertical transmission of SARS-CoV-2 can occur, and babies are more likely to be admitted to neonatal units compared with babies born to uninfected people [12–14]. When adverse perinatal outcomes arise, determinants include early gestational age at infection, pre-existing maternal comorbidities, higher maternal age, higher maternal body mass index, maternal ventilatory support, and low neonatal birthweight [12,15].

The Centers for Disease Control and Prevention states that, compared with the general population, pregnant people are at an increased risk for severe illness and death from COVID-19, and should protect themselves through measures such as social distancing, hand hygiene, and face...
coverings, and that they may choose to receive a COVID-19 vaccine [16]. Worldwide, there are between 1800 million (2013 estimate) and 1900 million (2025 estimate) females of reproductive age (15–49 years) [17]. The global unplanned pregnancy rate is approximately 44% of all pregnancies, varying between different geographies and socioeconomic groups [18], and so together with people who are knowingly pregnant, a large proportion of the global population may be clinically vulnerable to COVID-19 illness and in need of enhanced protection. Several vaccines against COVID-19 have been deployed globally in recent months [19–25]. One of these vaccines, AZD1222 (also known as ChAdOx1 nCov-19 and Vaxzevria) is a recombinant replication-deficient non-human adenovirus that encodes the highly immunogenic SARS-CoV-2 spike glycoprotein, which induces specific antibody and T cell responses [26, 27]. Available data do not indicate any harm to pregnancy but, to date, AZD1222 has not been authorized for use in pregnant or breastfeeding people. Information provided to healthcare professionals states that AZD1222 should only be considered in pregnancy when the potential benefits of vaccination outweigh any potential risks for the mother and fetus [23, 28].

To contribute to the overall safety profile of a vaccine intended for a population that includes pregnant and breastfeeding people, as well as people of reproductive age, developmental and reproductive toxicity studies conducted in accordance with European Medicines Agency, US Food and Drug Administration, World Health Organization (WHO) guidelines and International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) [29–32] are necessary. Speciﬁc selection for vaccine toxicity testing requires that the species is relevant [31], and in the case of AZD1222, that the species mounts an immune response following vaccination. In the present study, mice were chosen because they are commonly used in development and reproductive studies, in accordance with guidelines from the Centers for Disease Control and Prevention, US Food and Drug Administration, and WHO [29–31]. Importantly for the present study, AZD1222 induces robust antibody and cell-mediated immune responses in mice [27, 33], mice have previously been used to study HuCoV infection [34], and more recently have been used to model COVID-19 [35]. Furthermore, the methods to reliably measure immunogenicity in mice, and the industry-accepted protocols for necessary endpoints in mice, are available [32].

Reducing the transmission of SARS-CoV-2 to pregnant and breastfeeding people to protect them from COVID-19, potentially through vaccination, is an area of high unmet clinical need. To support the inclusion of pregnant and breastfeeding people in clinical studies with AZD1222, the aims of this non-clinical study were to: 1) evaluate the effects of intra-muscular administration of appropriate doses of AZD1222 on the fertility and reproductive processes of the female CD-1 mouse during the embryofetal development (EFD) phase, and on postnatal outcomes during the littering phase; and 2) measure immunogenicity responses in dams, fetuses, and pups following vaccination of dams.

2. Material and methods

2.1. Animals

Studies were conducted according to Good Laboratory Practice regulations for nonclinical laboratory studies and complied with ARRIVE guidelines. All studies used female CD-1 mice, who were approximately 11 weeks of age at the start of the study and were obtained from Charles River Laboratories (Charles River UK Limited, Margate, Kent, UK). Animal housing and care were compliant with the Guide for the Care and Use of Laboratory Animals (8th edition). All procedures were approved by the Home Office, United Kingdom, with adherence to the Animals (Scientific Procedures) Act 1986 at a facility with American Association for Accreditation of Laboratory Animal Care accreditation.

2.2. Test agent

AZD1222 (batch MS00684-92) with a virus particles concentration (VP) of $5.3 \times 10^{11}$/mL was the test agent. For control studies, A438 Buffer (35 mM sodium chloride, 10 mM histidine, 1 mM magnesium chloride, 0.1 mM ethylenediaminetetraacetic acid, 7.5% sucrose, 0.1% Tween 80, 0.5% ethanol) was used. For each adult female, 0.035 mL of agent was administered intramuscularly in the thigh muscle of each hindlimb on each dosing occasion (0.07 mL/occasion, thus $3.7 \times 10^{11}$...
animals were thoroughly examined after arrival and before the start of the study, once at the start and once towards the end of the working day. All

Table 1 ). Immunogenicity assessments were additionally made at specific points during each study phase.

| Study Group | Endpoint / Assessment | Timepoint |
|-------------|-----------------------|-----------|
| **Embryofetal development phase** | | |
| Dam | First dose | Day 1 (13 days before pairing) |
| | Second dose | GD 6 |
| | Serology (S-glycoprotein) | GD 6 |
| | Female fertility | GD 17.5 |
| F1 fetuses | Uterine observations | GD 17.5 |
| | Serology (S-glycoprotein) | GD 17.5 |
| | Embryofetal development (gross, visceral, head and skeletal examinations) | GD 17.5 |
| **Littering phase** | | |
| Dam | First dose | GD 6 |
| | Second dose | GD 15 |
| | Serology (S-glycoprotein) | GD15, PNDs 7 and 21 |
| | Parturition | PND 1 |
| | Serology (S-glycoprotein) | PNDs 7 and 21 |
| F1 progeny | Survival, growth | Ongoing to PND 21 |
| | Sexual maturation | Ongoing to PND 41–44 (selected offspring) |

* Prior to pairing.

**  At terminal anesthesia. GD, gestation day; PND, postnatal day.

VPs per dosing occasion in the test group).

2.3. Study design

The study protocol was developed in accordance with US Food and Drug Administration and WHO guidelines for assessing the potential toxicity of vaccines for infectious diseases [29–31], and conformed to the principles of ICH guidelines on pharmaceutical and biopharmaceutical development.

The study was split into two phases: 1) an EFD phase to provide information on potential effects of AZD1222 on female mating behavior, fertility, pregnancy, implantation, and embryofetal morphological development in utero; and 2) a littering phase that included postnatal assessments to provide information on potential effects of AZD1222 on parturition, pup survival, development, and sexual maturity (Fig. 1 and Table 1). Immunogenicity assessments were additionally made at specific points during each study phase.

EFD phase female mice were dosed with AZD1222 (n = 25) or control (n = 25) on day 1 (13 days prior to pairing for mating) and on gestation day (GD) 6. On GD 17.5, dams were terminally anesthetized and a cesarean section performed. After mating, littering phase dams were dosed with AZD1222 (n = 25) or control (n = 25) on GDs 6 and 15. Dams littered normally (with regular observation to check for difficulties during parturition) and were terminally anesthetized with unselected pups on postnatal day (PND) 21. Selected pups, hereon referred to as the F1 generation, were terminally anesthetized between PNDs 41 and 44. Male CD-1 mice were included in each study phase but were not dosed and were removed from the study after reviews of the mating and pregnancy rates in females.

2.4. In-life observations and measurements

Mortality and moribundity were checked twice daily throughout the study, once at the start and once towards the end of the working day. All animals were thoroughly examined after arrival and before the start of the dosing period and were given a detailed physical examination once during Week 1. EFD phase animals were then examined daily from day 1 when they received their first dose of vaccine or control. Littering phase dams were examined once weekly from day 1, and then daily from day 14. All were observed regularly throughout each day of dosing for potential vaccine-related reactions. The onset, intensity, and duration of any signs were recorded as appropriate, with particular attention paid during the first hour after dosing. For comparison, control animals were examined to the same schedule as the treated animals. Assessments included physical observations, bodyweights, food consumption, dermal scoring (assessment of the skin at the injection site for signs of erythema and eschar formation, edema formation, and any other reaction to treatment pre-dose and for 2 days following each dosing occasion), mating performance, fertility indices, duration of gestation, litter performance, litter survival indices, litter and pup weights, eye opening in pups, assessment of sexual maturation (selected pups), immunogenicity (dams, fetuses, and pups), gravid uterus weights and corrected maternal bodyweights (EFD phase only), examinations of pregnancies with fetal weights and examinations (external, visceral, and skeletal examination: EFD phase only), and gross pathology (dams and pups). In brief, EFD phase dams were terminally anesthetized on GD 17.5, a cesarean section was performed and the uterus was examined. Each fetus was identified and examined for visible external abnormalities. Pooled litter fetal blood samples were taken. Approximately half of all fetuses were then prepared for detailed head and brain examination using a modified Wilson’s technique [36]. All fetuses were examined for abnormalities of the internal organs (thoracic and abdominal cavities). Skeletal examination following alizarin red S preparation was performed on approximately 50% of fetuses to determine any skeletal malformations and the degree of ossification.

2.5. Immunogenicity analysis

Blood samples were collected at specific times for serological immunogenicity determination. A validated electrochemiluminescent immunoassay for the relative-quantification of anti-spike glycoprotein 1–OT##Author’s comment: ‘on the PDF proof, this word currently breaks over two lines - flow forward if possible’. Please check and paginate accordingly.-=>antibodies in murine serum was used. The sequential direct format assay used SARS-CoV-2 spike glycoprotein (AstraZeneca; GenBank QHD43416.1) immobilized on the solid phase to capture anti–spike glycoprotein immunoglobulins in murine serum samples, and a ruthenium-labeled goat anti-murine immunoglobulin G (MSD®) for detection. An anti–spike glycoprotein monoclonal antibody (AstraZeneca) was used as calibrator; data were reported in arbitrary units/mL (AU/mL) relative to the calibrator. The limits of quantification for the assay were 0.25–52.00 AU/mL.

Up to 0.25 mL of blood was collected from the saphenous vein, except for samples at terminal anesthesia, which were collected from the orbital sinus. EFD phase animals had samples taken prior to mating on day 14 (13 days after their first dose), GD 7 (the day after their boost dose), and GD 17.5 (at termination). Fetal samples, pooled per litter, were also taken on GD 17.5. Littering phase dams had samples taken on GD 15 (the day of
3. Results

3.1. Physical assessments

Throughout the course of the study, and in both the EFD and littering phases (Fig. 1), there were no treatment-related unscheduled deaths, physical signs (including no erythema or oedema at the injection sites on any dosing occasion), effects on pre-mating or maternal body weight gain (Fig. 2), or food consumption, in dams. Furthermore, following terminal anesthesia there were no gross pathology findings.

3.2. Mating and fertility performance

Results from both study phases show that there were no AZD1222-related effects on mating performance or fertility compared with control (Table 3). Across all groups, the majority of females (97%) showed a positive sign of mating within 7 nights of cohabitation. The remaining 3% showed no clear indication of mating but were found to be pregnant. The pregnancy rate (the proportion of females cohabiting with a male who became pregnant) was 94% in the AZD1222 groups, and 92% in the control groups.

3.3. Ovarian and uterine assessments

Assessments made during the EFD phase show that 84% of dams in the control group, and 96% of dams in the in the vaccine group, had live fetuses, with a mean of 14.2 fetuses per dam in each group. Gravid uterine weight, number of corpora lutea, pre- and post-implantation loss, and number of implantations were comparable between groups.

Table 2

| Variable for inferential analysis | Statistical method |
|----------------------------------|--------------------|
|                                  | Parametric | Non-parametric | Incidence |
| Body weight[^a^]                              | X         | NA            | NA        |
| Body weight gains[^a^]                        | X         | NA            | NA        |
| Food consumption[^a^]                        | X         | NA            | NA        |

[^a^] The two groups for each phase were compared using a Dunnett’s test (equivalent to t-test in Nevis 2012 tables).

[^b^] The two groups for each phase were compared using a Dunn’s test (equivalent to Wilcoxon Rank-Sum test in Nevis 2012 tables) if it was significant.

[^c^] A Fisher’s exact test was used to compare the two groups for each phase in each analysis. NA; not applicable; X, analyses were performed.

Fig. 2. Mean group body weight gain in dams. There were no treatment-related effects on pre-mating or maternal body weight gain in embryofetal development or littering dams. EFD, embryofetal development; GD, gestation day.
Results from both study phases show that there were no AZD1222-related effects on key indices of mating, fertility, pregnancy and parturition compared with control.

- Proportion of females cohabiting with a male who became pregnant.
- Number of days from GD 0 to the day the first pup was observed.
- Proportion of pregnancies that resulted in birth of live litters.
- Proportion of implants that resulted in pups.
- Proportion of pups born alive.
- Proportion of pups born that survived 4 days postpartum.
- Percentage of pups alive 4 days postpartum that were alive 14 days postpartum. EFD, embryofetal development; GD, gestation day.

### Table 4

| Assessment                              | Control | AZD1222 |
|-----------------------------------------|---------|---------|
| Gravid uterine weight, g                | 21.70 (3.63) | 21.99 (3.07) |
| Proportion of dams with live fetuses    | 100     | 100     |
| Number of corpora lutea                 | 15.9 (2.3) | 16.3 (2.8) |
| Number of implantations                 | 15.2 (2.5) | 15.4 (2.3) |
| % pre-implantation loss                 | 4.12 (4.54) | 5.00 (6.31) |
| % post-implantation loss rate           | 6.79 (10.02) | 7.45 (10.62) |
| Total resorptions                       | 1.0 (1.5)  | 1.2 (2.0)  |
| Early resorptions                       | 1.0 (1.5)  | 1.0 (1.9)  |
| Late resorptions                        | 0.0 (0.3)  | 0.2 (0.5)  |

Data are mean (standard deviation), or %.

There were no AZD1222-related effects on uterine examinations or litter observations compared with control on GD 17.5.

- Proportion of implants that did not result in a fetus.
- Early embryonic resorption (discrete, formless, discoloured tissue mass attached to the internal uterine wall: may be of varying size).
- Late embryonic resorption (macerated tissue identifiable as an embryo or fetus, with recognisable external features such as tail, limbs, mouth and nares present). EFD, embryofetal development; GD, gestation day.

### Table 5

| Examination type / classification | Control | AZD1222 |
|----------------------------------|---------|---------|
| External                         | Fetuses examined, n / evaluated, n | 298 / 298 |
|                                  | Litters examined, n / evaluated, n | 231 / 21 |
| Malformation\(^a\)               | Litter % of fetuses                | 6.2     |
|                                  | Litters, n                         | 1       |
| Fixed head                       | Fetuses examined, n / evaluated, n | 144 / 298 |
|                                  | Litters examined, n / evaluated, n | 21 / 21  |
| Incidental\(^a\)                 | Litter % of fetuses                | 3.27    |
|                                  | Litters, n                         | 2       |
| Variation\(^a\)                  | Litter % of fetuses                | 0.60    |
|                                  | Litters, n                         | 1       |
| Fixed visceral body              | Fetuses examined, n / evaluated, n | 144 / 298 |
|                                  | Litters examined, n / evaluated, n | 21 / 21  |
| Malformation\(^a\)               | Litter % of fetuses                | 17      |
|                                  | Litters, n                         | 1       |
| Variation\(^a\)                  | Litter % of fetuses                | 11.65   |
|                                  | Litters, n                         | 12      |
| Skeletal                         | Fetuses examined, n / evaluated, n | 154 / 298 |
|                                  | Litters examined, n / evaluated, n | 21 / 21  |
| Malformation\(^a\)               | Litter % of fetuses                | 9.62    |
|                                  | Litters, n                         | 9       |
| Variation\(^a\)                  | Litter % of fetuses                | 0.60    |
|                                  | Litters, n                         | 1       |
| Malformation\(^a\)               | Litter % of fetuses                | 17.9    |
|                                  | Litters, n                         | 15      |
| Variation\(^a\)                  | Litter % of fetuses                | 95.25   |
|                                  | Litters, n                         | 21      |
| Malformation\(^a\)               | Litter % of fetuses                | 1.79    |
|                                  | Litters, n                         | 1       |

Data are mean (standard deviation), or %.

### Table 3

| Assessment                              | Study phase | Control | AZD1222 |
|-----------------------------------------|-------------|---------|---------|
| Mean number of nights to positive      | EFD and littering | 2.4 | 3.0 |
| mating sign                             |             |         |        |
| Pregnancy rate, %                       | EFD and littering | 92 | 94 |
| Mean (standard deviation) fetal weight, g | EFD         | 1.06 (0.06) | 1.10 (0.11) |
| Male : female live fetal ratio, n EFD   | 7.1 : 7.0    | 7.2 : 7.0 |
| Mean gestation duration\(^a\), days     | Littering   | 18.9    | 19.0  |
| Gestation index\(^b\), %                | Littering   | 100     | 100   |
| Birth index\(^c\), %                   | Littering   | 91.2    | 92.6  |
| Live birth index\(^c\), %              | Littering   | 100     | 99.7  |
| Mean litter size, n                    | Littering   | 15.1    | 15.2  |
| Viability index\(^d\) days 0–4 %       | Littering   | 90.5    | 97.9  |
| Lactation index\(^e\) days 4–21 %      | Littering   | 99.7    | 99.7  |

### 3.4. Embryofetal development

Assessments made during the EFD phase show that, across both groups, the extent of pre- and post-implantation loss was similar and that the mean fetal weight was comparable between groups. Evaluations of fetal morphological development in utero revealed no differences in external, visceral, or skeletal observations between fetuses from AZD1222-vaccinated dams compared with controls (Table 5). The incidence and type of variations and malformations observed were generally comparable between the AZD1222 and the control groups, or were of insufficient incidence to be considered test item-related effects, or were comparable to the Test Facility historical control data set.

### 3.5. Gestation

Assessments made during the littering phase show that there were no AZD1222-related effects on the duration of gestation compared with control (Table 3), with a 100% gestation index (the proportion of pregnant dams producing a live litter) across both groups.

### 3.6. Parturition and pup survival

Littering phase data showed that there were no AZD1222-related effects on parturition or pup survival indices compared with control, including litter size, live birth index (the proportion of pups who were born alive), mean pup weight, viability index (the proportion of pups born alive who were alive on PND 4), and lactation index (the proportion of pups alive on PND 4 who were alive on PND 14). There were, furthermore, no vaccine-related abnormal observations for dams or pups during the lactation period (Table 3).
3.7. Postnatal development

Indices of pup development, including bodyweight gain and eye opening, were comparable between groups, regardless of whether the dam had received AZD1222 or control. Any minor differences were within the expected range of biological variation, and within the range of institutional historical controls for this type of study. Following terminal anesthesia of unselected pups on PND 21, there were no AZD1222-related findings in gross pathology.

3.8. Observations in the F1 generation after PND 21

There were no AZD1222-related effects on bodyweight, bodyweight gain, or food consumption in F1 progeny after PND 21, compared with controls. Indices of sexual maturation, namely age and weight at male preputial separation and female vaginal opening, revealed similarities between vaccine and control groups (Fig. 3). Following terminal anesthesia of pups between PNDs 41 and 44, there were no AZD1222-related findings in gross pathology.

3.9. Demonstration of seroconversion

All samples collected from EFD phase control animals, and all samples with one exception from littering phase control animals, were below the limit of quantification for the assay and were considered seronegative. The exception was a single sample collected on PND 21, which returned a result of 5.93 AU/mL, lower than any concentration obtained from samples collected from vaccinated animals. Together with the fact that this dam was seronegative on GD 15, as were pups from the dam’s litter on PND 21, this result suggests that cross-contamination may have occurred.

In EFD phase animals who received AZD1222, immunogenicity analyses indicated an antibody response to spike glycoprotein prior to pairing for mating, with seroconversion of all vaccinated animals occurring 14 days after the first dose of vaccine was administered on day 1. Antibody responses to spike glycoprotein were maintained from day 14 (mean [standard deviation, SD]: 344.42 [277.89] AU/mL) through GD 7, the day after the booster dose (751.99 [463.46] AU/mL). Dam and fetal samples were also seropositive on GD 17.5 (mean [SD]: 247.54 [288.48] and 78.90 [46.55] AU/mL, respectively) (Fig. 4a), providing evidence that seropositivity of dams was maintained throughout the gestation period, and that placental transfer of maternal immunoglobulins to fetuses occurred.

In littering phase dams who received AZD1222, immunogenicity analyses following a single dose of vaccine on GD 6 indicated an antibody response to spike glycoprotein, with seroconversion of all vaccinated dams by GD 15 (mean [SD] antibody concentration: 52.29 [30.39] AU/mL). Samples collected on PND 7 and PND 21, following the booster dose of vaccine on GD 15, showed an increased antibody response to spike glycoprotein compared with samples collected prior to this day (262.72 [370.59] and 419.32 [299.38] AU/mL, respectively). Pooled samples collected from pups on PND 21 were seropositive (120.34 [71.32] AU/mL).

These greater pup anti–spike glycoprotein antibody levels compared with fetal samples on GD 17.5, suggest pup exposure to maternal antibodies during lactation (Fig. 4b).

![Fig. 3. Assessment of sexual maturation in the F1 generation after postnatal day 21. For littering phase pups, there were no treatment-related effects on male age at preputial separation (A); male weight at preputial separation (B); female age at vaginal opening (C); and female weight at vaginal opening (D).](image-url)
4. Discussion

This nonclinical development and reproductive toxicity study in CD-1 mice supports the safety profile of AZD1222 by showing that intramuscular dosing of CD-1 female mice during the EFD phase on day 1 (13 days prior to pairing for mating) and on GD 6, or during the littering phase on GD 6 and GD 17.5, was well tolerated with the desired immunogenicity responses in dams and progeny. There was no evidence of AZD1222-related effects on female fertility, reproductive performance, gestation duration, fetal development, morphology or survival, parturition or postnatal outcomes, including pup survival, physical development, or sexual maturation. These results are comparable to and support those from an earlier dose-range finding developmental and reproductive toxicity study.

Providing passive protection to fetuses and neonates, whose immune systems are not fully developed, is important to protect against infection or to modify the severity of infectious diseases. In this study, vaccine-induced immunogenicity was evident in dams, fetuses, and pups following vaccination of the dam. Antibody responses to SARS-CoV-2 spike glycoprotein were raised in all dams following the first dose of AZD1222 and were maintained throughout the gestation and postnatal periods. Immunoglobulin levels in fetal serum at GD 17.5 suggest successful placental transfer of maternal antibodies in CD-1 mice following vaccination. The increased levels of pup immunoglobulin levels at PND 21 compared with GD 17.5 suggest effective lactational transfer of maternal antibodies. These modes of maternal antibody transmission align with what have previously been shown for mice [37].

Dams demonstrated a robust and consistent immunogenicity response following vaccination with AZD1222. No vaccine-related findings were seen in any of the parameters measured. Maternal vaccination with AZD1222 was not associated with any effects on female fertility, fetal, or pup development, or postnatal outcomes of pups. Male fertility was addressed in a separate study and was not associated with any effects of vaccination with AZD1222. The dose administered in this study ($3.71 \times 10^{10}$ VP on each occasion) exceeded the human dose on a mg/kg basis, and was the highest feasible dose that could be administered to mice via intramuscular injection, given the limitations of vaccine concentration and the total volume that could be administered given animal welfare considerations [31].

A study design consisting of two separate phases, with vaccination occurring prior to mating and again at implantation for one phase, and at implantation and again in late gestation for the other phase, allowed assessments of the potential effects of vaccination on fertility and embryofetal development, and on pre- and postnatal development of offspring, respectively. This design was in accordance with the international guidance on non-clinical studies to support vaccine development and aligns with the principles of developmental and reproductive toxicity assessments for pharmaceuticals set out by ICH guidelines.
study design utilized 25 animals per dosing group to ensure that sufficient litters and pups would result to allow meaningful interpretation of the data, with dam numbers aligned with recommendations provided by ICH guidance for investigations of this type [32]. This study demonstrated robust and consistent placental transfer of immunoglobulins induced by the vaccine from dam to fetus, as evidenced by fetal immunogenicity. Similarly, a robust and consistent lactational transfer of maternal immunoglobulins induced by the vaccine was demonstrated, as evidenced by pup immunogenicity. This is consistent with previous mouse vaccination studies (unrelated to COVID-19) which have shown efficient transfer of vaccine-induced maternal antibodies to the embryo/fetus and pup via the placenta and milk [38], although in rodents, antibodies may also be passively transferred via other routes [39].

Reducing the transmission of SARS-CoV-2 to pregnant and breastfeeding people to protect them from COVID-19 is an area of high unmet clinical need. Vaccination against SARS-CoV-2 is considered a relevant way to address this need provided it is safe for use in this population.

5. Conclusions

This non-clinical study demonstrates that AZD1222 has no adverse effects on female fertility, embryofetal development or postnatal development in mice. Furthermore, AZD1222 produced the desired antibody responses to SARS CoV-2 spike protein in both dams and progeny. These data, together with clinical data from non-pregnant people, support the inclusion of pregnant and breastfeeding people in clinical studies with AZD1222.

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Appendix A. Supplementary data
Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.reprotox.2021.07.010.

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