scientific reports

Check for updates

OPEN

Perinatal outcomes in RhD-negative pregnant women in Japan

Ken Takahashi[1]✉, Susumu Yoshida[2], Showa Aoki[3], Saori Tanaka[4], Akihiro Kawashima[5], Kazuhiro Kajiwara[6], Noriko Kato[7], Hitoshi Matsui[8], Mariko Serizawa[9], Shunichiro Tsuji[10], Tomoko Yamamoto[11], Tadatsugu Kinjo[12], Noriyuki Nakamura[13], Maiko Sagawa[14], Mika Sato[15], Emiko Abe[16], Sayuri Nakanishi[17], Yoshinobu Fujimoto[18], Sota Takahashi[19], Hiroyuki Sasaki[20], Yurika Mukai[21], Sumiko Hara[22], Kaori Fukuta[23], Norihiko Kikuchi[24], Emiko Hara[25], Tomomi Shiga[26], Chinami Horiuchi[27], Toshiyuki Sado[28], Yuko Matsubara[29], Keiichiroh Akabane[30], Aya Harada[31], Hiromi Nagase[32], Kazuhisa Maeda[33], Hiroshi Katagiri[34], Jun Sasahara[35], Hirokazu Sugii[36], Shunsuke Tamaru[37], Miyoko Waratani[38], Saya Tsukahara[39], Shohei Shibukawa[40], Tomoiki Kiyama[41], Yoshifumi Kasuga[42], Makiko Egawa[43], Hiroshi Sato[44], Chiyo Tamamura[45], Tokumasa Suemitsu[46], Aikou Okamoto[1] & Osamu Samura[1]

Managing RhD-negative pregnancies is vital for preventing hemolytic disease of the fetus and newborn, which occurs when RhD-negative mothers develop anti-D antibodies after exposure to RhD-positive fetal blood. This retrospective cohort study evaluated the proportion of RhD-negative pregnancies and newborns in Japan by assessing current management practices and outcomes. This study included RhD-negative pregnant women who delivered at 22 weeks or later at 47 Japanese facilities between April 2018 and March 2023. Pregnancies with unknown newborn RhD status were excluded. Data were obtained from medical records. Among the 1088 RhD-negative women, 1062 met the inclusion criteria. RhD-negative pregnancies comprised 0.71% of the total cohort, with 8.7% RhD-negative newborns. Anti-D immunoglobulin was administered in 96.5% of pregnancies, with a maternal spontaneous sensitization rate of 0.6% before 28 weeks and no sensitization detected from 28 weeks to postpartum. Sensitized RhD-negative women had higher cesarean section, preterm delivery, and neonatal hemolytic anemia rates than the non-sensitized group, leading to increased neonatal intensive care unit admissions. Despite the low incidence of RhD-negative pregnancies, this study underscores the need for tailored management strategies, suggesting that non-invasive prenatal diagnosis of fetal RhD status could prevent unnecessary anti-D immunoglobulin administration, improving outcomes and resource utilization in Japan.

Keywords RhD-negative pregnancies, Hemolytic disease of the fetus and newborn, Anti-D immunoglobulin, Maternal sensitization, Non-invasive prenatal diagnosis, Neonatal hemolytic anemia

[1]Department of Obstetrics and Gynecology, The Jikei University School of Medicine, 3-25-8 Nishi-Shimbashi, Minato-ku, Tokyo 105-8461, Japan. [2]Department of Obstetrics and Gynecology, Toyonaka Municipal Hospital, 4-14-1 Shibahara-cho, Toyonaka-shi, Osaka 560-8565, Japan. [3]Department of Obstetrics and Gynecology, Uji-Tokushukai Medical Center, 145 Ishibashi, Makijima-cho, Uji, Kyoto 611-0041, Japan. [4]Department of Obstetrics and Gynecology, San-ikukai Hospital, 3-20-2 Taihei, Sumida-ku, Tokyo 130-0012, Japan. [5]Department of Obstetrics and Gynecology, Showa University School of Medicine, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8666, Japan. [6]National Center for Child Health and Development, 2-10-1, Okura 2-chome, Setagaya-ku, Tokyo 157-8535, Japan. [7]Department of Obstetrics and Gynecology, Japanese Red Cross Aichi Medical Center Nagoya Daini Hospital, 2-9, Myoko-cho, Showa-ku, Nagoya 466-8650, Japan. [8]Department of Obstetrics and Gynecology, Chigasaki City Hospital, 5-15-1 Honmura, Chigasaki City, Kanagawa 253-0042, Japan. [9]Department of Obstetrics and Gynecology, Hamamatsu Medical Center, 328 Tomizuka-cho, Chuo-ku, Hamamatsu-shi, Shizuoka 432-8580, Japan. [10]Department of Obstetrics and Gynecology, Shiga University of Medical Science, Seta Tsukinawa-cho, Otsu City, Shiga 520-2192, Japan. [11]Department of Obstetrics and Gynecology, Faculty of Medicine, Saga University, 5-1-1 Nabeshima, Saga City, Saga Prefecture 849-8501, Japan. [12]Department of Obstetrics and Gynecology, University of the Ryukyus Hospital, 207 Aza-Uehara, Nishihara-cho, Nakagami-gun, Okinawa 903-0215, Japan. [13]Department of Obstetrics and Gynecology, Anjo Kosei Hospital, 28 Higashi-Hirokute, Anjo-cho, Anjo, Aichi 446-8602, Japan. [14]Department of Obstetrics and Gynecology, NHO Kure Medical Center and Chugoku Cancer Center, 3-1 Aoyama-cho, Kure City, Hiroshima 737-0023, Japan. [15]Department of Obstetrics and Gynecology, Chiba University Hospital,

1-8-1, Inohana, Chuo-ku, Chiba-shi, Chiba 260-8677, Japan. [16]Department of Obstetrics and Gynecology, Ehime Prefectural Central Hospital, 83 Kasuga-cho, Matsuyama, Ehime 790-0024, Japan. [17]Perinatal Center for Maternity and Neonate, Yokohama City University Medical Center, 4-57 Urafune-cho, Minami-ku, Yokohama 232-0024, Japan. [18]Department of Obstetrics and Gynecology, Hiratsuka City Hospital, 1-19-1 Minamihara, Hiratsuka-shi, Kanagawa 254-0065, Japan. [19]Department of Obstetrics and Gynecology, Hachinohe City Hospital, 3-1-1 Tamukai, Hachinohe 031-8555, Japan. [20]Department of Obstetrics and Gynecology, Saiseikai Yokohamashi Tobu Hospital, 3-6-1 Shimosueyoshi, Tsurumi-ku, Yokohama, Kanagawa 230-8765, Japan. [21]Department of Obstetrics and Gynecology, Hiroshima University Hospital, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan. [22]Department of Obstetrics and Gynecology, Tokyo Adventist Hospital, 3-17-3 Amanuma, Suginami-ku, Tokyo 167-8507, Japan. [23]Department of Obstetrics and Gynecology, Kouseiren Takaoka Hospital, 5-10 Eiraku-cho, Takaoka-shi, Toyama 933-8555, Japan. [24]Department of Obstetrics and Gynecology, Shinshu University Hospital, 3-1-1 Asahi, Matsumoto-shi, Nagano 390-8621, Japan. [25]Department of Obstetrics and Gynecology, Kitakyushu Municipal Medical Center, 1-1, Maben 2-chome, Kokurakita-ku, Kitakyushu 802-8561, Japan. [26]Department of Obstetrics and Gynecology, Gifu University Hospital, 1-1 Yanagido, Gifu City, Gifu Prefecture 501-1194, Japan. [27]Department of Obstetrics and Gynecology, Sakakibara Heart Institute, 3-16-1 Asahi-cho, Fuchu-shi, Tokyo 183-0003, Japan. [28]Department of Obstetrics and Gynecology, Nara Prefectural General Medical Center, 2-897-5 Shichijo Nishi-machi, Nara City, Nara 630-8581, Japan. [29]Department of Obstetrics and Gynecology, Ehime University Hospital, 454 Shitsukawa, Toon-shi, Ehime 791-0295, Japan. [30]Department of Obstetrics and Gynecology, Hokkaido University Hospital, Kita 14 Nishi 5, Kita-ku, Sapporo 060-8648, Japan. [31]Department of Obstetrics and Gynecology, Japan Baptist Hospital, 47 Kitashirakawa Yamanomoto-cho, Sakyo-ku, Kyoto 606-8273, Japan. [32]Department of Obstetrics and Gynecology, Kanagawa Children's Medical Center, 2-138-4 Mutsukawa, Minami-ku, Yokohama-shi, Kanagawa 232-8555, Japan. [33]Department of Obstetrics and Gynecology, Shikoku Medical Center for Children and Adults, 2-1-1 Senyu-cho, Zentsuji City, Kagawa 765-8507, Japan. [34]Department of Obstetrics and Gynecology, Masuda Red Cross Hospital, 103-1 Otoyoshichoi, Masuda City, Shimane 698-8501, Japan. [35]Department of Obstetrics and Gynecology, Osaka Women's and Children's Hospital, 840 Murodo-cho, Izumi, Osaka 594-1101, Japan. [36]Department of Obstetrics and Gynecology, NHO Iwakuni Clinical Center, 1-1-1 Atago-cho, Iwakuni-shi, Yamaguchi 740-8510, Japan. [37]Department of Obstetrics and Gynecology, Saitama Medical University Hospital, 38 Morohongo, Moroyama-cho, Iruma-gun, Saitama 350-0495, Japan. [38]Department of Obstetrics and Gynecology, Kyoto Prefectural University of Medicine Hospital, 465 Kaji-cho, Hirokoji-agaru, Kawaramachi-dori, Kamigyo-ku, Kyoto 602-8566, Japan. [39]Department of Obstetrics and Gynecology, NHO Okayama Medical Center, 1711-1 Tamasu, Kita-ku, Okayama 701-1192, Japan. [40]Department of Obstetrics and Gynecology, Matsue Red Cross Hospital, 200 Moromachi, Matsue City, Shimane 690-8506, Japan. [41]Department of Obstetrics and Gynecology, Tottori Prefectural Kosei Hospital, 150 Higashishowa-machi, Kurayoshi City, Tottori Prefecture 682-0804, Japan. [42]Department of Obstetrics and Gynecology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan. [43]Department of Nutrition and Metabolism in Cardiovascular Disease, Graduate School of Medical and Dental Sciences, Institute of Science Tokyo, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519, Japan. [44]Department of Obstetrics and Gynecology, Hyogo Prefectural Amagasaki General Medical Center, 2-17-77, Higashinaniwa-cho, Amagasaki 660-8550, Japan. [45]Department of Obstetrics and Gynecology, University of Fukui Hospital, 23-3, Matsuoka-Shimo-Aizuki, Eiheiji-cho, Yoshida-gun, Fukui 910-1193, Japan. [46]Department of Obstetrics and Gynecology, Kameda Medical Center, 929 Higashi-cho, Kamogawa-shi, Chiba 296-0041, Japan. ✉email: ms01.takahashike@gmail.com

RhD-negative individuals lack the D antigen on the surface of their erythrocytes. Consequently, exposure to the D antigen through allogeneic blood transfusion or pregnancy with an RhD-positive fetus can lead to the production of anti-D antibodies[1]. These antibodies are of particular concern during pregnancy since they can cause severe fetal hemolytic disease and even fetal death. Anti-D immunoglobulin is commonly administered during pregnancy and postpartum worldwide to prevent the formation of anti-D antibodies[1]. However, non-invasive methods using maternal blood currently allow for the avoidance of anti-D immunoglobulin administration if the fetus is predicted to be RhD-negative[2–4]. These methods, which are widely used in Europe, employ quantitative polymerase chain reaction to detect the presence or absence of the RHD gene in RhD-negative pregnant women, particularly in those with homozygous deletions of the RHD gene. This approach is effective in Caucasians, where most RhD-negative cases involve such deletions. However, introducing these methods into clinical practice has been challenging in East Asian countries, such as Japan, China, and South Korea, where RhD-negative cases usually involve complex RHD gene polymorphisms. In previous studies, we successfully addressed these genetic differences by conducting polymorphism analysis using next-generation sequencing, enabling the prenatal diagnosis of fetal RhD status from maternal blood in East Asian countries, including Japan[5,6].

The demand for prenatal diagnosis using fetal RhD blood varies substantially across races and regions. RhD-negative cases occur in approximately 15%, 8%, and < 1% of Caucasians, Africans, and East Asians, respectively[7]. Regionally, the prevalence is approximately 13.9% and 4.7% in the United States and the Uyghur Autonomous Region, respectively[8]. A higher percentage of RhD-negative individuals generally correlates with a greater demand for prenatal fetal RhD blood group testing. Under these circumstances, the frequency of RhD-negative individuals in East Asia, including Japan, is the lowest worldwide. Specifically, the frequency of RhD-negative Japanese individuals is reported to be considerably low at approximately 0.5%[9], resulting in a lower demand for prenatal RhD diagnosis than in Western countries, where such testing is more commonly used in clinical applications. Assuming Hardy–Weinberg equilibrium within the Japanese population, the estimated proportion of RhD-negative newborns born to RhD-negative mothers is approximately 1 in 14–15[5]. However, precise data on this frequency are lacking. Therefore, the need for fetal RhD prenatal diagnosis in Japan requires careful evaluation before widespread clinical adoption.

In Japan, the United States, and other countries, anti-D human immunoglobulin is administered once during pregnancy to RhD-negative women to prevent anti-D antibody sensitization[10,11]. Previous studies have reported that this approach's efficacy in preventing sensitization during pregnancy is adequate[12]. However, McBain et al. highlighted that the risk of anti-D antibody sensitization may vary depending on the genotype of RhD-negative pregnant women[13]. The sensitization status and risk in racial populations with serologically diverse RhD-negative genotypes, such as the Japanese[14], are unknown. Additionally, the Practice Bulletin of the American College of Obstetricians and Gynecologists in 2017 reported that 40% of newborns born to RhD-negative pregnant women were RhD-negative[11]. However, the frequency of RhD-negative newborns in Japan is believed to be low, raising concerns about whether the same management strategies for populations with a high likelihood of conceiving an RhD-positive child are appropriate for those with a substantially low probability. In Japan, the standard management for RhD-negative pregnant women involves conducting an indirect Coombs test in the first trimester, at approximately 28 weeks of pregnancy, and postpartum to monitor maternal anti-D antibody sensitization. If no sensitization is detected, anti-D immunoglobulin (250 μg) is administered after the 28-week examination and re-administered postpartum[10]. However, whether this approach is appropriate for populations with different probabilities of conceiving RhD-positive children remains unexplored.

Therefore, this study assessed the proportion of RhD-positive and RhD-negative newborns born to RhD-negative pregnant women in Japan with the goal of evaluating the usefulness of the fetal RhD blood type prediction method using maternal blood that we developed. We sought to evaluate the frequency of RhD-negative pregnancies, anti-D immunoglobulin administration practices, and sensitization status of RhD-negative pregnant women as the secondary outcome. Moreover, we hypothesized and tested the hypothesis that sensitized RhD-negative women have a considerably higher incidence of perinatal complications than non-sensitized RhD-negative women.

Results

Proportion of RhD-negative pregnant women and RhD-negative newborns

During the study period, 152,526 eligible women gave birth at the participating centers. Among these, 1,088 were RhD-negative pregnant women, and 26 were excluded from the analysis due to unknown RhD blood types in their newborns. The total number of newborns was 1,091, with 996 RhD-positive and 95 RhD-negative newborns (Fig. 1). Additionally, the proportions of RhD-negative women among all pregnancies and RhD-negative newborns born to RhD-negative pregnant women were 0.71%, and 8.7%, respectively.

Anti-D human Immunoglobulin administration status and complications

The median gestational age at the time of anti-D human immunoglobulin administration was 28 (interquartile range: 28–28) weeks. Among the 1,053 women (excluding 9 with missing data from the 1062 women), 96.5%

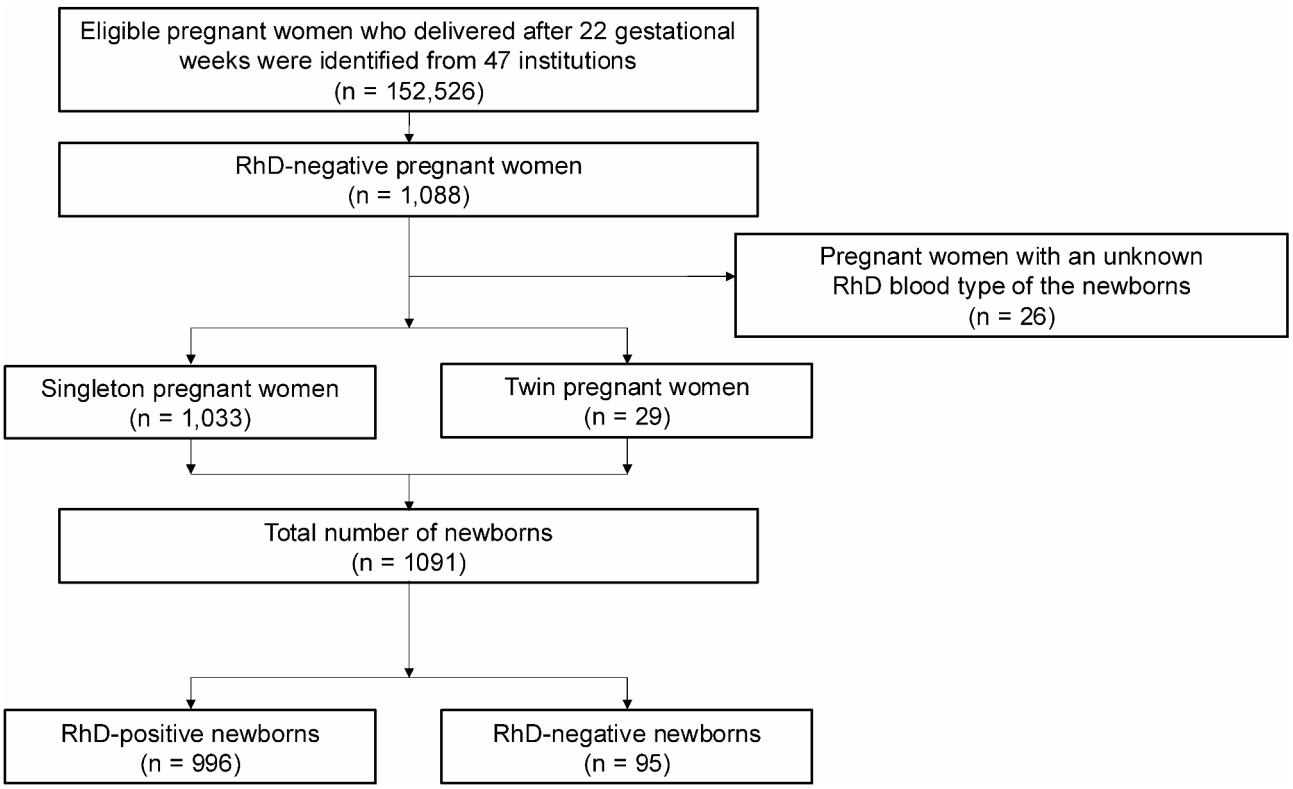


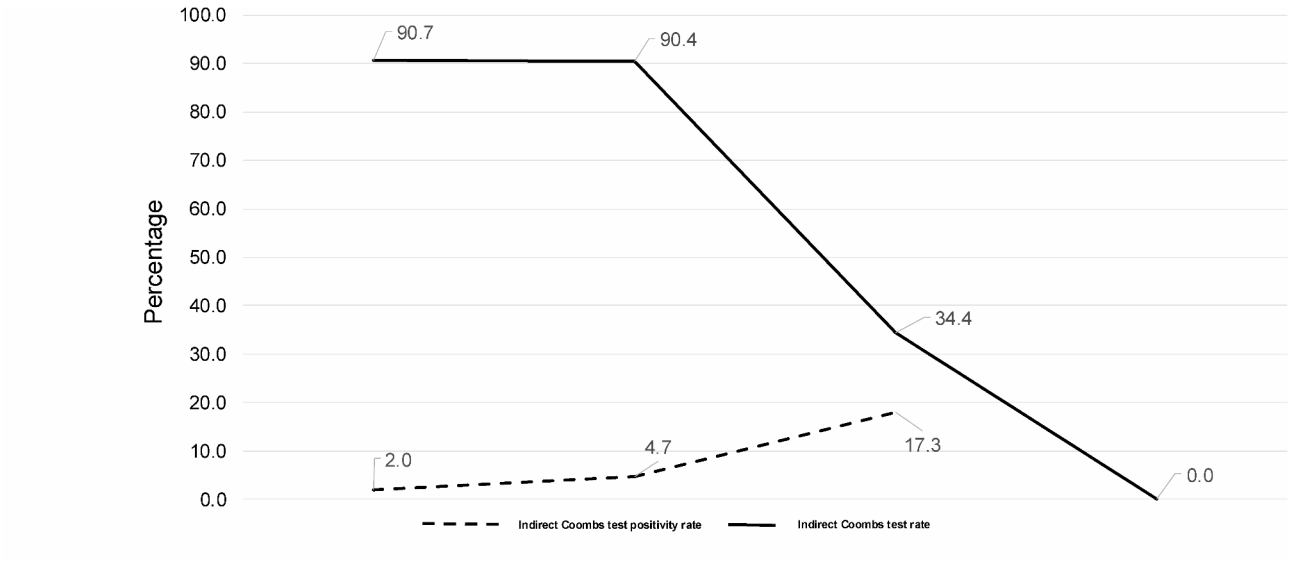
Fig. 1. Study flow chart. Among the 152,526 pregnant women who delivered after 22 weeks at 47 facilities, 1088 were RhD-negative. Ninety-five of the 1091 newborns were RhD-negative.

(1,016/1,053) were administered anti-D human immunoglobulin, whereas 3.5% (37/1,053) were not. Proper and incorrect administrations were observed in 99.8% (1014/1016) and 0.2% (2/1,016) of the anti-D prophylaxis administration cases, respectively. Both cases of incorrect administration included women who were already sensitized. Among the women not administered anti-D human immunoglobulin, 62.2% (23/37) had valid reasons for not receiving the anti-D prophylaxis, namely previous maternal sensitization to anti-D antibodies or having a partner who was RhD-negative. In contrast, 37.8% (14/37) did not receive it due to inappropriate reasons, including the lack of prenatal checkups or failure to receive the necessary administration. The rate of postpartum anti-D prophylaxis administration was 89.4% (937/1,048) in the group who received the treatment but 10.6% (111/1,048) in the group that did not receive it. Notably, 1,048 individuals were included after excluding 14 with missing data from the 1,062 women. Among the administered individuals, 99.3% (930/937) and 0.7% (7/937) were correctly and incorrectly administered, respectively. Incorrect administrations occurred in four cases where the newborn was RhD-negative and three where the mother had already been sensitized. Among the individuals who did not receive the treatment, 98.2% (109/111) were correctly identified and not administered the prophylaxis due to the fetus being RhD-negative or the mother having preexisting sensitization to RhD antibodies. However, 1.8% (2/111) did not receive the administration mistakenly. This included one case where vaccination was missed following a hysterectomy performed due to massive postpartum hemorrhage and one where administration was forgotten. Postpartum anti-D prophylaxis was administered within 72 h in 99.9% (936/937) of cases. Data on adverse reactions were obtained for 1,032 patients after excluding cases with missing information on adverse reactions from those who received anti-D prophylaxis. Among them, adverse reactions, including hypotension and gastrointestinal symptoms, were observed in two patients. One patient with gastrointestinal symptoms required pharmacological treatment.

Indirect coombs test status and results

Figure 2 shows the results of the indirect Coombs test and its positivity rate. During the first trimester, 90.7% (946/1,043) of pregnant women underwent an indirect Coombs test, whereas approximately 10% did not. Specifically, 1043 represents the total of 1062 individuals after excluding 19 with missing data. The reasons for not taking the test included forgetting or not receiving a prenatal checkup. During the indirect Coombs test performed at approximately 28 weeks of gestation, 90.4% (943/1,043) of pregnant women were tested, while approximately 10% were not. The total of 1043 represents the 1062 individuals after excluding 19 with missing data. Postpartum indirect Coombs test uptake was notably low at 34.4% (364/1,058), and the uptake rate at 1 month postpartum was 0%. The total number 1,058 represents the 1,062 individuals after excluding 4 with missing data.

Furthermore, the positivity rates for the indirect Coombs test at different periods were found in 2.0% (19/946) of patients in the first trimester, of whom 17 had pre-existing anti-D antibodies; 4.7% (44/943) at approximately 28 weeks of gestation; and 17.3% (63/364) postpartum (Fig. 2). Indirect Coombs tests performed at approximately 28 weeks of gestation and immediately postpartum showed many positive results due to residual anti-D prophylaxis. Many cases exist where anti-D prophylaxis was administered due to genital bleeding



Implementation gestational weeks (interquartile range) or time	10 weeks (9–12)	28 weeks (26–28)	Postpartum	1 month postpartum
Indirect Coombs test results Positive (anti-D immunoglobulin)/Negative/No inspection/Missing data	19(0)/927/97/19	44(17)/899/100/19	63(55)/301/694/4	0(0)/0/1,062/0

Fig. 2. Status and results of irregular antibody testing. More than 90% of cases were tested in the first trimester and at approximately 28 weeks of gestation; however, the proportion dropped substantially postpartum. No cases were tested after 1 month postpartum. The results of the indirect Coombs test were notably affected by anti-D human immunoglobulin administration.

Implementation gestational weeks (interquartile range) or time	10 weeks (9–12)		28 weeks (26–28)		Postpartum
Mothers with anti-D antibody sensitization	17	+5	22	+0	22
Rate of natural sensitization to anti-D antibody during this period (Number of sensitized pregnant women/Total number of eligible pregnant women)		+0.6% (5/850)		+0.0% (0/347)	

Fig. 3. Maternal anti-D antibody sensitization status. Some cases of spontaneous sensitization to anti-D antibodies during pregnancy have occurred, although the proportion is relatively small. Specifically, 850 represents the number of patients who underwent the indirect Coombs test at approximately 10 and 28 weeks of gestation, with available results. In contrast, 347 represents the number of patients who underwent the indirect Coombs test immediately postpartum and at approximately 28 weeks of gestation, with available results.

	Pregnant women with anti-D antibody sensitization ($n = 22$)	Pregnant women without anti-D antibody sensitization ($n = 1040$)	p-value*
Gravidity (including the current pregnancy)	3 (2–3)	2 (1–3)	< 0.001
Parity (including the current pregnancy)	1 (1–2)	0 (0–1)	< 0.001
History of maternal blood transfusions (prior to current pregnancy)	15.0 (3/20)	1.6 (16/1,026)	0.003
Fetal anemia during pregnancy	27.3 (6/22)	0.0 (0/1,038)	< 0.001
Fetal transfusions during pregnancy	13.6 (3/22)	0.0 (0/1,038)	< 0.001
Maternal treatment during pregnancy	13.6 (3/22)	0.0 (0/1,040)	< 0.001
Gestational weeks at delivery (median)	37.5 (34.75–38.25)	39 (38–40)	< 0.001
Cesarean section	59.1 (13/22)	28.8 (299/1,039)	0.002

Table 1. Background information and perinatal outcomes of RhD-negative pregnant women. Data are presented as the number or median (interquartile range) or percentage (number of applicable women/number of women targeted for analysis). The number of participants is calculated by excluding the missing data from the total number of each group. *Wilcoxon signed-rank sum test, Fisher's exact test, or χ^2 test.

before the indirect Coombs test at 28 weeks of gestation or where it was administered early before the indirect Coombs test, although the details are unknown. Figure 3 illustrates the maternal antibody sensitization status based on anti-D antigen. At the time of testing in the first trimester, 1.8% (17/946) of RhD-negative pregnant women had anti-D antibody sensitization. Between the first trimester and 28 weeks of gestation, five cases were spontaneously sensitized to anti-D antibodies. No new sensitization to anti-D antibodies was observed from 28 weeks of gestation until immediately postpartum. The proportion of women sensitized to anti-D antibodies was 0.6% (5/850) from the first trimester to 28 weeks of gestation and 0.0% (0/347) from 28 weeks of gestation to the postpartum period. A larger-than-expected number of pregnant women did not undergo the indirect Coombs test, particularly during the postpartum period. Since maternal sensitization to anti-D antibodies cannot be determined immediately postpartum, it is ideally assessed at least 1 month postpartum. However, our study revealed that such evaluations are not routinely performed in Japan.

Background information and perinatal outcome of RhD-negative pregnant women

Table 1 presents the maternal background and perinatal outcomes of pregnant women sensitized to anti-D antibodies ($n = 22$) and those not sensitized ($n = 1,040$). The risk of anti-D sensitization was considerably elevated among women with more pregnancies and deliveries, as well as a history of blood transfusions. Additionally, the rates of fetal anemia, fetal blood transfusion, and maternal treatment during pregnancy were considerably higher in the sensitized group than in the non-sensitized group. However, this is expected, as these conditions occur and require intervention only in the sensitized group. The sensitized group had significantly earlier deliveries (37.5 vs. 39.0 weeks, $p < 0.001$) and a higher rate of cesarean Sect. (59.1 vs. 28.8%, $p = 0.002$) than the non-sensitized group.

Neonatal outcome

Table 2 presents the outcomes of children born to anti-D-sensitized ($n = 22$) and non-anti-D-sensitized ($n = 1069$) mothers. No substantial difference was observed in birth weight; however, birth height was considerably lower in the sensitized group than in the non-sensitized group. Although the pH levels of cord blood gas did not differ substantially, the Apgar scores at 1 and 5 min were considerably lower in the sensitized group than in the

non-sensitized group. Since the median values were similar between the two groups, whether this statistical significance has clinical relevance or if it is merely attributable to the sample size remains uncertain. Additionally, the incidence of hemolytic anemia in neonates (59.1 vs. 1.0%, $p < 0.001$) and the rate of admission to the neonatal intensive care unit (72.7 vs. 21.1%, $p < 0.001$) were significantly higher in the sensitized group than in the non-sensitized group.

Treatment of anti-D antibody-sensitized pregnant women and their newborns

Table 3 outlines the treatment status of the 22 pregnant women and their fetuses or neonates sensitized to anti-D antibodies. Fetal anemia was diagnosed in 27.3% ($n = 6$) of the cases, with 13.6% ($n = 3$) receiving fetal transfusions. It was identified through elevated peak systolic velocity in the middle cerebral artery in six cases, four of which also required fetal blood sampling. Maternal treatment included plasma exchange therapy in 13.6% ($n = 3$) of cases; one showed a considerable response, whereas two showed no response. Among the 22 newborns, 59.1% ($n = 13$) had hemolytic anemia. The treatments administered for hemolytic anemia were phototherapy, gamma-globulin, plasma exchange, and exchange transfusion in 100% ($n = 13$), 18.2% ($n = 4$), 18.2% ($n = 4$), and 0.5% ($n = 1$), respectively. No neonatal deaths were reported.

Discussion

This study assessed the proportion of RhD-negative pregnant women in Japan, proportion of RhD blood groups among infants born to these women, management of RhD-negative pregnancies, and perinatal outcomes and treatment status of anti-D antibody-sensitized mothers. Our results revealed that 0.7% of pregnant women are RhD-negative, and 8.7% have RhD-negative infants. This indicates that approximately 1 in 11 RhD-negative pregnant women receive unnecessary anti-D immunoglobulins during pregnancy. Although the overall management of RhD-negative pregnancies in Japan is generally effective and appropriate, instances of inaccurate management have occurred. In populations such as the Japanese population, where fetuses are more likely to be RhD-positive, the risk of anti-D sensitization is elevated, making appropriate management even more crucial. Anti-D immunoglobulin is generally safe; however, it must be administered with caution due to the potential for adverse reactions, although these are rare. Pregnancies with anti-D antibody sensitization are high-risk for the mother and child and require careful medical intervention during pregnancy and postpartum.

The prevalence of RhD-negative individuals varies substantially according to their genetic background, suggesting differences across race, region, and country. Consequently, the value and utility of fetal RhD prenatal diagnosis also vary widely based on race and region. In Switzerland, approximately 36% of RhD-negative pregnant women had RhD-negative fetuses, and prenatal diagnosis of RhD blood type has been reported to be useful[15]. In the present study, only 8.7% of RhD-negative pregnant women had RhD-negative fetuses. Although the usefulness of prenatal diagnosis may be lower in the Japanese population than in the Caucasian population, it can still benefit some pregnant women by preventing unnecessary anti-D immunoglobulin administration. The cost-effectiveness of implementing prenatal diagnosis in Japan warrants further investigation. However, prenatal diagnosis may be cost-effective in this context, given the substantial reduction reported in the cost of analysis using next-generation sequencers[16].

Although obstetric care in Japan generally follows established obstetric guidelines[10], the management of RhD-negative pregnant women was found to be highly accurate. Most RhD-negative pregnant women underwent an indirect Coombs test at the appropriate time, and anti-D immunoglobulin was administered when necessary. One measure for effective management is preventing anti-D sensitization during pregnancy. In the present study, no cases of anti-D sensitization were observed from 28 weeks of gestation to the postpartum period. A previous study reported that administering anti-D immunoglobulin at 28 weeks of gestation reduces the sensitization rate from 2–0.2%[17]; however, our results suggest that management based on current obstetric guidelines is effective

	Neonates born to mothers sensitized to anti-D antibodies ($n = 22$)	Neonates born to mothers not sensitized to anti-D antibodies ($n = 1,040$)	p-value*
Neonatal birth weight (g)	2,769 ± 558	2921 ± 492	0.155
Neonatal birth height (cm)	47.2 ± 3.1	48.6 ± 3.0	0.025
Sex (male, female)	54.5, 45.5 (12/22, 10/22)	51.9, 48.1(555/1,069, 514/1,069)	0.807
Neonatal blood type (A, B, AB, O)	50.0, 18.2, 4.5, 27.3 (11/22, 4/22, 1/22, 6/22)	37.5, 21.2, 11.3, 30.0 (399/1,065, 26/1,065, 120/1065, 320/1,065)	0.589
Umbilical artery blood gas pH	7.28 ± 0.11	7.30 ± 0.06	0.166
Apgar score at 1 min	8 (8–8)	8 (8–8)	0.009
Apgar score at 5 min	9 (8–9)	9(9–9)	0.007
Hemolytic anemia	59.1 (13/22)	1.0 (11/1,053)	< 0.001
Morphological abnormalities	4.5 (1/22)	6.1 (65/1,064)	1
NICU admission rate	72.7 (16/22)	21.1 (225/1,066)	< 0.001

Table 2. Neonatal outcome of RhD-negative pregnant women. Data are presented as the mean ± standard deviation or median (interquartile range) or percentage (number of applicable women/number of women targeted for analysis). The number of participants is calculated by excluding the missing data from the total number of each group. *NICU* neonatal intensive care unit. *Wilcoxon signed-rank sum test, Fisher's exact test, or Student's t-test.

Case	Anti-D immunoglobulin administration during pregnancy	Anti-D antibody titer in indirect coombs test			Anti-D immunoglobulin administration postpartum	Irregular antibodies other than anti-D	Fetal anemia	Diagnostic basis for fetal anemia	Fetal transfusion	Maternal treatment	Mode of delivery	Delivery weeks of gestation	Hemolytic anemia of the newborn	Anti-D antibodies test in neonates	Neonatal treatment
		1st trimester	Approximately 28 weeks of gestation	Postpartum											
1	–	×1	×2	No inspection	–	–	–	N/A	–	–	CS	36	–	Negative	–
2	–	Negative	×64	×64	–	–	–	N/A	–	–	CS	37	+	Positive	P
3	–	×1	×1	×18	–	Anti-C	–	N/A	–	–	V	38	–	Negative	–
4	–	Negative	×4	No inspection	+	–	–	N/A	–	–	CS	38	+	Positive	P
5	–	×2048	×512	No inspection	–	–	+	E, FBS	+	–	CS	37	+	Positive	P, ET
6	–	Negative	Positive (antibody titer unknown)	No inspection	–	–	–	N/A	–	–	V	38	+	Positive	P
7	–	×1024	×1024	No inspection	–	–	+	E, FBS	+	–	CS	33	+	Positive	P
8	+	×2	×2	No inspection	+	Anti-C	+	E	–	–	V	38	+	Positive	P
9	–	×2	×	No inspection	–	–	–	N/A	–	–	V	39	–	Negative	P
10	–	Negative	×4	No inspection	–	–	–	N/A	–	–	CS	38	–	Negative	–
11	+	×128	×128	No inspection	–	–	–	N/A	–	–	CS	37	+	Positive	P, G
12	+	Positive (Antibody titer unknown)	×32	No inspection	+	–	–	N/A	–	–	V	39	–	Positive	–
13	–	×256	×16	×2	–	–	–	N/A	–	PE	V	40	–	No inspection	–
14	–	Negative	×32	Positive (Antibody titer unknown)	–	–	–	N/A	–	–	CS	36	–	No inspection	–
15	–	×4	×32	×2048	–	Anti-C	–	N/A	–	–	V	39	–	Negative	P
16	–	×512	×256	No inspection	–	Anti-C	–	N/A	–	–	V	39	–	Negative	P
17	–	×16	×64	No inspection	–	Anti-C	+	E, FBS	–	–	CS	34	+	Positive	P, G
18	–	×64	×2,048	×16,384	–	Anti-C, Anti-Jkb, Anti-Dia	+	E, FBS	+	–	CS	34	+	Positive	P, G, PE
19	–	×8	×128	No inspection	–	Anti-C	–	N/A	–	–	V	38	+	Positive	P, PE
20	–	×64	×16	No inspection	–	–	–	N/A	–	–	CS	35	+	Positive	P
21	–	×2	×128	×2048	–	–	+	E	–	PE	CS	33	+	Positive	P, G, PE
22	–	×2	×512	No inspection	–	–	–	N/A	–	PE	CS	33	+	Positive	PE

Table 3. Treatment of anti-D antibody-sensitized pregnant women and their newborns. *CS* cesarean section, *E* elevated middle cerebral artery peak systolic velocity, *ET* exchange transfusion, *FBS* fetal blood sampling, *G* gamma-globulin therapy, *N/A* not applicable, *P* phototherapy, *PE* plasma exchange, *V* vaginal.

even for populations such as the Japanese population, who have a higher proportion of RhD-positive fetuses and are more susceptible to sensitization. Spontaneous sensitization to anti-D antibodies occurred in 0.6% of patients between early pregnancy and 28 weeks of gestation. These findings suggest that the optimal number of doses during pregnancy should be reconsidered.

In the present study, no fetal or neonatal deaths were observed among pregnant women sensitized to anti-D antibodies. However, several risks have been identified, including an increased cesarean section rate, a higher risk of preterm delivery, the occurrence of hemolytic anemia in newborns, and a considerable rise in neonatal intensive care unit admission rates. These factors represent substantially high risks for perinatal management. The management of anti-D antibody-positive pregnant women in Japan[10] involves several key measures, such as antibody titer measurement and fetal ultrasound, to monitor the fetus's condition. A higher antibody titer correlates with an increased risk of fetal anemia and may necessitate medical interventions, such as fetal blood transfusion or exchange transfusion for the newborn[18]. The risk is particularly high when the antibody titer exceeds 1,000 times[19].

In this study, all cases with antibody titers exceeding 1,000 during pregnancy were diagnosed with fetal anemia and subsequently received fetal blood transfusions. A considerable number of infants also undergo exchange transfusions or plasma exchange during the neonatal period. However, cases of fetal anemia with antibody titers < 1,000 and those requiring intensive neonatal treatment were also observed.

Fetal anemia is primarily assessed using fetal ultrasound. Although anti-D antibody titers are crucial for managing pregnant women with anti-D sensitization, they are not sufficient by themselves. A comprehensive evaluation, including fetal ultrasound and other methods, is essential. If prenatal diagnosis of fetal RhD blood type using maternal blood is feasible, it could enable additional assessments beyond traditional evaluations of the mother and infant. This might include assessing the risk of fetal anemia during pregnancy and monitoring for increases in antibody titers. Therefore, prenatal RhD diagnosis is important in this context.

This study has some limitations. Although this was a multicenter study, the number of RhD-negative patients was relatively small. Additionally, the study was retrospective, with clinical data extracted from medical records by collaborators. Some data were missing due to unrecorded information or other issues with the medical records. The missing data and relatively small number of participants may slightly reduce the reliability of this study's results. Therefore, prospective studies are necessary to obtain more accurate data. Finally, this study faced challenges in terms of comprehensively investigating the race and place of origin of RhD-negative pregnant women and their spouses. Consequently, only a small number of non-Japanese individuals may have been included.

Despite these limitations, this study involved 47 participating centers across Japan and provided valuable insights into the frequency, management status, and issues related to RhD-negative pregnant women. It also provides important data on neonatal RhD blood groups and perinatal outcomes. This study is particularly important since it is the first to suggest that prenatal diagnosis of fetal RhD blood type could be beneficial in Japan. Based on this study's results, we should promote research and establish a system for the clinical application of prenatal diagnosis of fetal RhD blood type using maternal blood in Japan.

In conclusion, this study provides valuable insights into the frequency of RhD-negative pregnant women in Japan, proportion of RhD-negative newborns, and current challenges in managing RhD-negative pregnancies. It also examines the perinatal outcomes for RhD-negative pregnant women and their children and highlights the potential benefits of prenatal diagnosis of fetal RhD blood type. Our findings revealed that 1 in 11 RhD-negative pregnant women in Japan received unnecessary anti-D human immunoglobulin administration, indicating that accurate prenatal diagnosis can help avoid such unnecessary procedures. Integrating prenatal diagnosis into current management practices for RhD-negative pregnant women may also enhance the appropriateness of their care. Therefore, further research on the implementation of fetal RhD blood group diagnosis in Japan is warranted.

Methods

Study design and data sources

This multicenter, retrospective, observational cohort study included facilities accredited by the Japan Society of Perinatal and Neonatal Medicine-accredited Specialist Training Facilities. Among the 358 facilities, 47 consented to participate and were included in the study. This study focused on RhD-negative women who gave birth at one of the participating facilities between April 2018 and March 2023. The inclusion criteria were RhD-negative pregnant women aged ≥ 20 years who agreed to participate and underwent delivery management, including stillbirths, at a participating facility during the study period, with both singleton and twin pregnancies. Pregnant women who did not meet the above criteria, those > 22 weeks pregnant, those who refused to participate, and those with unknown RhD blood types for their newborns were excluded.

The following information was collected from the medical records:

- Total number of deliveries and the number of deliveries involving RhD-negative pregnant women during the relevant period.
- Status of anti-D human immunoglobulin administration and any associated complications, including whether it was **administered** or unknown, and whether complications occurred. If complications were present, the symptoms and how they were handled were identified.
- Indirect Coombs test status and results during the first trimester, at approximately 28 weeks of gestation, postpartum, and the 1-month checkup, including whether the test was performed. If the test was positive, the type of antibody and antibody titer were also confirmed.
- Background information and perinatal outcomes of RhD-negative pregnant women.
- Neonatal outcomes which are the results tested and evaluated by the pediatrician postpartum.

- Treatment of anti-D antibody-sensitized pregnant women and their newborns, that is, the specific treatment administered by the pediatrician to the newborns.

Perinatal outcomes were categorized based on anti-D antibody sensitization status into sensitized and non-sensitized groups. The sensitized and non-sensitized groups included RhD-negative pregnant women who were sensitized and not sensitized to anti-D antibodies before or during pregnancy, respectively. Indirect Coombs test results were attributed to globulin rather than sensitization if the antibody titer was less than four-fold after anti-D human immunoglobulin administration[20].

Management procedures for RhD-negative pregnant women in Japan

The Japanese obstetric practice guidelines describe the handling of RhD-negative pregnant women as follows[10]:

(i) An indirect Coombs test should be performed in the first trimester of pregnancy, at approximately 28 weeks of gestation, and immediately postpartum to confirm the presence of anti-D antibodies.
(ii) If the pregnant woman is anti-D antibody negative, the following tests and procedures should be performed to prevent anti-D antibody sensitization to the mother.

- Administer anti-D immunoglobulin (250 μg) at approximately 28 weeks of gestation.
- If the neonate is RhD positive, administer anti-D immunoglobulin (250 μg) to the mother within 72 h of delivery, but do not administer if the neonate is RhD negative.
- Cases of pre-existing sensitization should not be treated with anti-D human immunoglobulin.

If the anti-D antibody titer is less than four-fold postpartum, the patient is considered sensitization-free. A retrospective evaluation of compliance with the abovementioned procedures was conducted using medical records.

Study outcomes

The proportion of RhD-positive and RhD-negative newborns born to RhD-negative pregnant women was the primary endpoint. In contrast, the secondary endpoints were the frequency of RhD-negative pregnancies among women in Japan, administration status and complications related to anti-D human immunoglobulin, status and results of indirect Coombs tests, background information on RhD-negative pregnancies, perinatal outcomes for RhD-negative women and their newborns, and management of anti-D antibody-sensitized pregnant women and their newborns.

Ethics declarations

This study was conducted in an opt-out format, allowing patients to refuse participation instead of requiring explicit consent. Informed consent was obtained from all participants and/or their legal guardians through this opt-out process. This study protocol was approved by the Ethics Committee of Jikei University School of Medicine through a central batch application (approval number: 35–188) and those of all participating institutions. It was performed in accordance with the ethical standards outlined in the Declaration of Helsinki and the Japanese ethical guidelines.

Statistical analyses

After assessing the data distribution, Student's t-test was used to analyze normally distributed continuous variables, with results reported as means and standard deviations. Non-normally distributed continuous variables are presented as medians and interquartile ranges and were analyzed using the Wilcoxon signed-rank test. Categorical variables, such as preterm birth rate, cesarean section rate, sex, and blood type, were compared between the two groups using the χ^2 or Fisher's exact test, with results expressed as percentages. Statistical significance was set at $p < 0.05$. Missing data for each item were excluded from the analysis. No statistical sample size calculations were performed owing to the retrospective design of this study. All statistical analyses were conducted using Excel spreadsheets (Microsoft Corporation, Redmond, WA, USA) or JMP 9.0.2 (SAS Institute Japan).

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Received: 25 September 2024; Accepted: 13 March 2025
Published online: 22 March 2025

References

1. de Haas, M., Finning, K., Massey, E. & Roberts, D. J. Anti-D prophylaxis: past, present and future. *Transfus. Med.* **24**, 1–7 (2014).
2. Manfroi, S. et al. Prenatal non-invasive foetal RHD genotyping: diagnostic accuracy of a test as a guide for appropriate administration of antenatal anti-D immunoprophylaxis. *Blood Transfus.* **16**, 514–524 (2018).
3. de Haas, M. et al. Sensitivity of fetal RHD screening for safe guidance of targeted anti-D Immunoglobulin prophylaxis: prospective cohort study of a nationwide programme in the Netherlands. *BMJ* **355**, i5789. https://doi.org/10.1136/bmj.i5789 (2016).
4. Tynan, J. A. et al. Multiplexed analysis of Circulating cell-free fetal nucleic acids for noninvasive prenatal diagnostic RHD testing. *Am. J. Obstet. Gynecol.* **204**, 251e1–251e6 (2011).
5. Takahashi, K. et al. Amplicon sequencing-based noninvasive fetal genotyping for RHD-positive D antigen-negative alleles. *Clin. Chem.* **65**, 1307–1316 (2019).

6. Hori, A. et al. Improved library Preparation protocols for amplicon sequencing-based noninvasive fetal genotyping for RHD positive D antigen-negative alleles. *BMC Res. Notes* **14**, 380. https://doi.org/10.1186/s13104-021-05793-4 (2021).
7. Reid, M. E. & Lomas-Francis, C. P. Blood group system. In *The Blood Group Antigen Facts Book*. 105–108 (Elsevier Academic, 2004).
8. Yin, Q. & Flegel, W. A. DEL in China: the D antigen among serologic RhD-negative individuals. *J. Transl Med.* **19**, 439. https://doi.org/10.1186/s12967-021-03116-6 (2021).
9. Nussbaum, R. L., Mclnnes, R. R. & Willard, H. F. *Thompson & Thompson Genetics in Medicine* 8th edn (Elsevier, 2015).
10. Itakura, A. et al. Guidelines for obstetrical practice in Japan: Japan society of obstetrics and gynecology and Japan association of obstetricians and gynecologists 2020 edition. *J. Obstet. Gynaecol. Res.* **49**, 5–53 (2023).
11. Committee on Practice Bulletins-Obstetrics. Practice bulletin 181: prevention of RhD alloimmunization. *Obstet. Gynecol.* **130**, e57–e70 (2017).
12. Zonneveld, R. et al. Frequency and characterization of RHD variants in serologically D- Surinamese pregnant women and D-newborns. *Transfusion* **59**, 2672–2677 (2019).
13. McBain, R. D., Crowther, C. A. & Middleton, P. Anti-D administration in pregnancy for preventing Rhesus alloimmunization. *Cochrane Database Syst. Rev.* CD000020 https://doi.org/10.1002/14651858.CD000020.pub3 (2015).
14. Ogasawara, K. et al. Molecular basis for D- Japanese: identification of novel DEL and D- alleles. *Vox Sang* **109**, 359–365 (2015).
15. Schimanski, B. et al. Fetal RHD screening in RH1 negative pregnant women: experience in Switzerland. *Biomedicines* **11**, 2646. https://doi.org/10.3390/biomedicines11102646 (2023).
16. The Cost of Sequencing a Human Genome. https://www.genome.gov/about-genomics/fact-sheets/Sequencing-Human-Genome-cost
17. Bowman, J. M. Controversies in Rh prophylaxis. Who needs Rh immune Globulin and when should it be given? *Am. J. Obstet. Gynecol.* **151**, 289–294 (1985).
18. Tang, T. H. et al. Effect of Anti-D titers in RhD-negative pregnant women on fetuses and newborns: a retrospective study. *Pediatr. Neonatol.* **65**, 288–292 (2024).
19. Gottvall, T. & Hildén, J. O. Concentration of anti-D antibodies in Rh(D) alloimmunized pregnant women, as a predictor of anemia and/or hyperbilirubinemia in their newborn infants. *Acta Obstet. Gynecol. Scand.* **76**, 733–738 (1997).
20. Leveno, K. J. et al. Williams obstetrics (25th edition). (ed. Cunningham, FG) (New York: McGraw-Hill Medical, 2014).

Acknowledgements

The authors would like to thank Editage for English language editing and all participants in the study. Michishita Kaho, Tabe Midori, Satono Ueki, Mouri Shin, Shinsuke Tokoro, Miho Okamoto, Natsuko Takano, and Kihira Chikara substantially assisted in the data collection.

Author contributions

K.T. was involved in project development, data collection, and manuscript writing. O.S. was involved in data collection, project development, and manuscript writing. The following co-authors were instrumental in the collection of the data: S.Y., S.A., S.T., A.K., K.K., N.K., H.M., M.S., S.TS., T.Y., T.K., N.N., M.S., M.SA., E.A., S.N., Y.F., S.TA., H.S., Y.M., S.H., K.F., N.KI., E.H., T.S., C.H., T.SA., Y.M., A.K., A.H., H.N., K.M., H.K., J.S., H.SU., SH.TA., M.W., SA.TS., SH.SH, T.KI., Y.K., M.E., H.S., C.T., T.SU., and A.O. All authors read and approved the final manuscript.

Funding

This work was supported by the National Center for Child Health and Development of Japan (Grant Number 2023B-8), Japan Society for the Promotion of Science KAKENHI (Grant Number JP23K15847), and Japan Society of Perinatal and Neonatal Medicine (2022 Japan Society of Perinatal and Neonatal Medicine Clinical Research Award).

Declarations

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to K.T.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.