Effects of use of conventional and sexed semen on the conception rate in heifers: a comparison study

Kohei Oikawa[a], Takeshi Yamazaki[b], Satoshi Yamaguchi[c], Hayato Abe[c], Hanako Bai[a], Masashi Takahashi[a], Manabu Kawahara[a†]

[a] Laboratory of Animal Genetics and Reproduction, Research Faculty of Agriculture, Hokkaido University, Kita-ku Kita 9 Nishi 9, Sapporo 060-8589, Japan.

[b] Dairy Cattle Group, Division of Dairy Production Research, Hokkaido Agriculture Research Centre, NARO, Sapporo 062-8555, Japan.

[c] Computing Section, Milk Recording Division, Hokkaido Dairy Milk Recording and Testing Association, Sapporo 060-0004, Japan

[†] Corresponding Author:
Manabu Kawahara, Ph.D.
Laboratory of Animal Genetics and Reproduction, Research Faculty of Agriculture
Hokkaido University, Kita-ku Kita 9 Nishi 9, Sapporo 060-8589, Japan
E-mail: k-hara@anim.agr.hokudai.ac.jp
Tel. & Fax: +81-11-706-2541

Abstract

Conception rate with the use of sexed semen is lower than that with the use of conventional semen, posing a major problem in the dairy industry. The aim of this study was to understand the risk factors that affect the conception rate after artificial insemination (AI) with conventional and sexed semen using field data. The records of the first insemination in Holstein heifers with conventional (*n* = 41,857) and sexed semen (*n* = 45,465) in Hokkaido, Japan were analyzed. The mean conception rate after AI from 2012 to 2016 was 56.9% with conventional semen and 47.3% with sexed semen. A multivariable logistic regression model including the effects of year, heifer age, time of the year, semen type, service sire, and their interactions was used to evaluate the interaction effect of heifer age and time of the year by semen type on the conception rate. In the analysis using heifer age, we found that heifers inseminated with sexed semen were approximately 21 days younger than those inseminated with conventional semen. Interestingly, in early, warmer months (Jun, Jul, and Aug), the conception rate after AI with sexed semen significantly decreased compared with that after AI with conventional semen ($P < 0.01$). Our results showed that more careful implementation of AI is required for a stable conception using sexed semen, particularly during warmer months.

Keywords

Artificial insemination; Conception rate; Holstein heifer; Heat stress; Sexed semen

1. Introduction

Currently, in the dairy industry, sperm sexing is an essential reproductive technology that can control the sex ratio of offspring [1, 2]. X-Chromosome-bearing sperm is used for producing genetically desirable replacement heifers and thereby contributing to more efficient genetic improvement [3, 4]. Therefore, predetermination of offspring sex enables the dairy industry to promote the efficient production of females and the consequential increase in profit margins.

Sperm sorting using flow cytometry is the most reliable method for selecting X-chromosome-bearing sperm [1, 2]. Flow cytometry methods for sperm sorting has approximately 90% accuracy [5, 6]; however, it has detrimental effects on sperm viability [7]. Furthermore, a low number of sperms is packed in a sexed-semen straw due to the sorting efficiency of flow cytometry [8]. Consequently, the conception rate after artificial insemination (AI) with sexed semen is approximately 70%–80% of that with conventional semen in heifers [9] and cows [6]. Due to this low efficiency, the use of sexed semen is recommended for the first and second insemination of nulliparous heifers because of relatively high conception rates [6, 9]. Moreover, because of the low conception rates, sexed semen is frequently applied to fertile females displaying clear signs of estrus and high performance as candidates for breeding stock [5].

To improve the conception rate following AI with sexed semen, it is necessary to precisely understand the factors that affect the AI conception rate with conventional and sexed semen. Although several field studies have reported the factors that affect the conception rate after AI with sexed semen [5, 10], information on the effects of each factor is limited. For instance, the effects of time of the year and heifer age on the AI conception rate with conventional and sexed semen remain unclear. Thus, the aim of this study was to understand the difference between conventional and sexed semen by comparing the effect of risk factors (heifer age and time of the year) on the conception rate after AI.

2. Materials and methods

2.1 Data

The AI records, with either conventional or sexed semen, of commercial Holstein herds in Hokkaido (located at 41°21′–45°33′N latitude and 139°20′–148°53′E longitude), Japan were collected through the Dairy Herd Improvement Program and were obtained from the Hokkaido Dairy Milk Recording and Testing Association (Sapporo, Japan). The available data for analysis included birth date of heifer, insemination date, semen code, and delivery date. To limit confounding effects from differences in fertility, milk production, uterine and ovarian recovery, endocrine system, and metabolic alterations, we focused on the AI records of nulliparous heifers. Furthermore, to limit the confounding effect by insemination number, we focused on the AI records of the first insemination. The records of AI using imported semen were excluded, because we could not determine whether the imported semen was conventional or sexed based on the semen identification codes. The data that included more than five heifer records in the contemporary service sire groups were analyzed. The conception rate was defined as a binary trait of 0 or 1, where 1 indicated that the first insemination attempt successfully achieved pregnancy and 0 indicated otherwise. In this study, the success or failure of insemination was judged from the insemination and delivery records. Finally, the data included 41,857 and 45,465 AI records for conventional and sexed semen, respectively.

2.2 Statistical Analysis

All analyses were performed using the computing environment R [11]. In most routine national evaluations, binary data of fertility traits have been based on linear models [12, 13]. However, theoretically, the threshold or logistic model is appropriate for the analysis of binary data [14, 15]. Therefore, we applied a logistic regression model in this study. Initially, the full model was developed including the effects of year, heifer age, time of the year, semen type, service sire, and all possible interactions. Then, non-significant effects ($P < 0.1$) were removed from the model using a backward elimination procedure, and the following logistic regression model was determined:

$$logit(\pi) = \mu + b_{1i}YEAR_i + b_{2j}AGE_j + b_{3k}MONTH_k + b_4SEMEN + b_{5m}Sire_m + b_{6j}AGE_j \times SEMEN + b_{7k}MONTH_k \times SEMEN$$

where, π is the probability of a successful pregnancy and $logit(\pi) = ln\ (\pi / 1 - \pi)$ is the link function; μ is the intercept of the model expressing the reference animal, that is, a heifer of the first group (year: 2012; age: ≤ 12 months; time of the year: Jan) and inseminated with conventional semen of the first sire; $YEAR_i$ is an indicator variable with the value of 1 when insemination was performed in year i with four groups (2013 through 2016), AGE_j is an indicator variable with the value of 1 when insemination was performed at age j with nine groups (13, 14, 15, 16, 17, 18, 19, 20, and ≥ 21 months); $MONTH_k$ is an indicator variable with the value of 1 when the insemination was performed at month k with 11 groups (calendar month except for Jan); *SEMEN* is an indicator variable with the value of 1 when the sexed semen was used for insemination; $Sire_m$ is an indicator variable with the value of 1 when the semen used for insemination was from sire m with 146 groups; $AGE_j \times SEMEN$ is an indicator variable with the value of 1 when insemination was performed at age j with sexed semen; $MONTH_k \times SEMEN$ is an indicator variable with the value of 1 when insemination was performed at month k with sexed semen. To explore the interaction effects of heifer age and time of the year by semen type on the conception rate, the odds ratio and 99% confidence interval (CI) were calculated using this logistic regression model.

3. Results

3.1 Factors affecting the conception rate

To evaluate the factors affecting the conception rate, variances of the effect in the model were analyzed (Table 1). The effect of year, heifer age, time of the year, semen type, and service sire were all significant ($P < 0.01$). Furthermore, the interaction effect of heifer age and time of the year by semen type was significant ($P < 0.05$). These results suggested that the effects of heifer age and time of the year on the conception rate after AI differed between conventional and sexed semen.

3.2 Status of use and conception rate after AI with conventional and sexed semen from 2012 to 2016

To investigate the status of use of sexed semen, the semen types (conventional and sexed semen) used for AI from 2012 to 2016 were determined (Table 2). The use of conventional semen for AI consistently decreased from 64.4% in 2012 to 35.3% in 2016. In contrast, the use of sexed semen for AI increased from 35.6% in 2012 to 64.7% in 2016. These results show that the use of sexed semen increased as the use of conventional semen decreased for AI in dairy heifers.

From 2012 to 2016, the mean of raw conception rate was 56.9% with conventional semen and 47.3% with sexed semen (Table 2). The conception rate with sexed semen was approximately 80% of that with conventional semen. With both semen types, the raw conception rates increased from 2012 to 2016.

3.3 Effect of heifer age on the conception rate after AI using conventional or sexed semen

The number of AI attempts for each heifer age group was investigated (Table 3). Heifers inseminated with sexed semen were approximately 21 days younger than those inseminated with conventional semen; the mean ± standard deviation was 438.5 ± 60.1 and 459.5 ± 76.2 days, respectively.

To investigate the interaction effect of heifer age and semen type on the conception rate, the raw conception rate and odds ratio for heifer age ranging from ≤ 12 months to ≥ 21 months were calculated (Table 3). With conventional semen, the raw conception rate gradually increased with the age of heifers (≤ 12: 54.8%; ≥ 21: 64.6%), but not with sexed semen. From the comparison of 99% CIs of two semen types within each age, the decrease in conception rate after AI with sexed semen was significant at 14 months and ≥ 21 months ($P < 0.01$).

3.4 Effect of time of the year on the conception rate after AI using conventional and sexed semen

The number of AI attempts in each time of the year was assessed (Table 4). The number of AI attempts was the highest in Jun with both the semen types (conventional: 4,479; sexed: 5,045).

To explore the interaction effect of the time of the year and semen type on the conception rates, we investigated the raw conception rate and odds ratio at each month of insemination from Jan to Dec with both the semen types (Table 4). From the comparison of 99% CIs of two semen types within each time of the year, the decrease in conception rate after AI with sexed semen was significant in Feb and early, warmer months (Jun, Jul, and Aug). In addition, we investigated the differences between the mean conception rate throughout the study period and for each month (Supplemental Figure S1). In early, warmer months, the decreases in conception rate after AI with sexed semen was higher than that with conventional semen. This suggests that heat sensitivity differed between AI with conventional and sexed semen.

4. Discussion

Using sexed semen for AI is essential for the practical control of sex ratio of offspring, which enables genetic improvement; however, the conception rate following AI with sexed semen remains lower than that following AI with conventional semen [6, 9]. Understanding the difference between conventional and sexed semen can lead to the achievement of stable conception rates following AI using sexed semen. In this study, we compared the effects of both heifer age and time of the year at insemination on the conception rate after AI with conventional and sexed semen using field data.

From 2012 to 2016, the raw conception rate increased with the use of both the semen types. To increase the fertility of semen in a straw for AI, a new method of preparing straw was developed, which was applied for sexed semen straw from 2011 in a Japanese company [16]. This technology might have contributed to the increase in the conception rate after AI with sexed semen, particularly from 2012 to 2013.

In the analysis using heifer age, we found that the age of heifer inseminated with sexed semen was 21 days younger than that of heifer inseminated with conventional semen. This result was consistent with that of a previous study showing that heifer age at calving was 26 days younger for AI with sexed semen than that with conventional semen [5]. This might be because of the selective use of sexed semen for genetically superior, fertile females, which are candidates for breeding stock. Generally, farmers determine the time of first insemination in heifers based on the following maternal physiological characteristics: age, weight, and height. Therefore, fully-grown heifers of younger age are considered desirable breeding stock, because the non-productive period is minimized by conceiving at a younger age [17]. The results of the present study reflect that younger, fully grown heifers tended to be inseminated to a greater extent with sexed semen rather than with conventional semen.

Next, we analyzed the effect of time of the year on the conception rate following AI with conventional and sexed semen. The highest number of AI attempts was observed in Jun with both the semen types. Cows that are pregnant in Jun would produce calves in early spring, which ensures the maximum utilization of nutritious spring pasture and leads to the highest productivity in pasture-based dairy production systems [18]. Therefore, the decline in the conception rate in Jun with the use of both types of semen might be related to excessive AI attempts, because the increase in the number of AI attempts is directly related to frequent AI at the wrong time, resulting in a decrease in the conception rate.

Finally, we investigated the conception rate in each month from Jan to Dec following AI with conventional and sexed semen. Strikingly, in early, warmer months (Jun, Jul, and Aug), the conception rate after AI with sexed semen significantly decreased compared to that with conventional semen. The conception rate in warmer months is relatively lower than that in other months in dairy cows [19]. It is well known that heat stress impairs reproductive performances in artificially inseminated cows. For instance, the duration and intensity of estrus was reduced in heat-stressed heifers [20, 21]. Luteolysis was also delayed in heat-stressed heifers [22]. Additionally, hotter environments impaired the developmental competency of bovine embryos [23-25]. However, damage to both heifers and embryos from hotter environments was common for AI using both the semen types, which did not appear to be a direct cause of decreased conception rate specific to AI using sexed semen in warmer months.

Sexed semen has a potentially reduced sperm lifespan in the female genital tract [26], and has lower sperm numbers per AI straw than that in conventional semen [8]. In addition, the developmental competency of sexed semen-derived embryo after fertilization was inferior to that of conventional semen-derived embryo *in vivo* [27] and *in vitro* [28]. Therefore, AI with sexed semen requires more precise timing of insemination and more careful handling [29]. Although further studies are needed to address why the conception rate after AI with sexed semen is low in relatively warmer months (Jun, Jul, and Aug), these sexed semen-specific features might reflect the higher sensitivity to hotter environments of sexed semen than that of conventional semen.

5. Conclusions

The effects of heifer age and time of the year on the conception rate after AI differed between conventional and sexed semen. The age of heifer inseminated with sexed semen was 21 days younger than that inseminated with conventional semen, which might reflect the selective use of sexed semen for genetically superior, fertile females. Meanwhile, the conception rate following AI with sexed semen significantly decreased compared with that with conventional semen in relatively early, warmer months (Jun, Jul, and Aug). This discrepancy is possibly explained by the following sexed semen-specific features: reduced sperm lifespan, lower sperm numbers in an AI straw, and lower embryo quality. Thus, our results highlight the need for more careful AI implementation considering that the timing of insemination would improve the conception rate after AI using sexed semen, particularly during warmer months.

Acknowledgments

All authors contributed to the interpretation of the data and read and approved the final manuscript.

Funding

This work was supported by a Grant-in-Aid for Scientific Research (B) to Manabu K. [grant number 18H02321] from the Japan Society for the Promotion of Science.

Declarations of interest: None

References

[1] Garner DL, Gledhill BL, Pinkel D, Lake S, Stephenson D, Van Dilla MA, et al. Quantification of the X- and Y-chromosome-bearing spermatozoa of domestic animals by flow cytometry. Biol Reprod. 1983;28:312-21.

[2] Johnson LA, Flook JP, Hawk HW. Sex preselection in rabbits: live births from X and Y

sperm separated by DNA and cell sorting. Biol Reprod. 1989;41:199-203.

[3] Seidel GE. Economics of selecting for sex: the most important genetic trait. Theriogenology. 2003;59:585-98.

[4] De Vries A, Overton M, Fetrow J, Leslie K, Eicker S, Rogers G. Exploring the impact of sexed semen on the structure of the dairy industry. J Dairy Sci. 2008;91:847-56.

[5] DeJarnette JM, Nebel RL, Marshall CE. Evaluating the success of sex-sorted semen in US dairy herds from on farm records. Theriogenology. 2009;71:49-58.

[6] Norman HD, Hutchison JL, Miller RH. Use of sexed semen and its effect on conception rate, calf sex, dystocia, and stillbirth of Holsteins in the United States. J Dairy Sci. 2010;93:3880-90.

[7] Rath D, Moench-Tegeder G, Taylor U, Johnson LA. Improved quality of sex-sorted sperm: a prerequisite for wider commercial application. Theriogenology. 2009;71:22-9.

[8] Seidel GE. Overview of sexing sperm. Theriogenology. 2007;68:443-6.

[9] Healy AA, House JK, Thomson PC. Artificial insemination field data on the use of sexed and conventional semen in nulliparous Holstein heifers. J Dairy Sci. 2013;96:1905-14.

[10] Cerchiaro I, Cassandro M, Dal Zotto R, Carnier P, Gallo L. A field study on fertility and purity of sex-sorted cattle sperm. J Dairy Sci. 2007;90:2538-42.

[11] R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/. 2017.

[12] Mark T. Applied genetic evaluations for production and functional traits in dairy cattle. J

Dairy Sci. 2004;87:2641-52.

[13] Jamrozik J, Fatehi J, Kistemaker GJ, Schaeffer LR. Estimates of genetic parameters for Canadian Holstein female reproduction traits. J Dairy Sci. 2005;88:2199-208.

[14] Gianola D. Theory and analysis of threshold characters. Journal of Animal Science. 1982;54:1079-96.

[15] Guo G, Hou Y, Zhang Y, Su G. Comparison of models for genetic evaluation of number of inseminations to conception in Danish Holstein cows. Anim Sci J. 2017;88:567-74.

[16] Livestock Improvement Association of Japan. https://patents.justia.com/patent/20130267771.

[17] Wathes DC, Pollott GE, Johnson KF, Richardson H, Cooke JS. Heifer fertility and carry over consequences for life time production in dairy and beef cattle. Animal. 2014;8 Suppl 1:91-104.

[18] Dillon P, Crosse S, Stakelum G, Flynn F. The effect of calving date and stocking rate on the performance of spring-calving dairy cows. Grass and Forage Science; 1995. p. 286-99.

[19] Hagiya K, Hayasaka K, Yamazaki T, Shirai T, Osawa T, Terawaki Y, et al. Effects of heat stress on production, somatic cell score and conception rate in Holsteins. Anim Sci J. 2017;88:3-10.

[20] Gwazdauskas FC, Thatcher WW, Kiddy CA, Paape MJ, Wilcox CJ. Hormonal patterns during heat stress following PGF(2)alpha-tham salt induced luteal regression in heifers. Theriogenology. 1981;16:271-85.

[21] De Rensis F, Scaramuzzi RJ. Heat stress and seasonal effects on reproduction in the dairy

cow--a review. Theriogenology. 2003;60:1139-51.

[22] Wilson SJ, Kirby CJ, Koenigsfeld AT, Keisler DH, Lucy MC. Effects of controlled heat stress on ovarian function of dairy cattle. 2. Heifers. J Dairy Sci. 1998;81:2132-8.

[23] Sakatani M, Alvarez NV, Takahashi M, Hansen PJ. Consequences of physiological heat shock beginning at the zygote stage on embryonic development and expression of stress response genes in cattle. J Dairy Sci. 2012;95:3080-91.

[24] Gendelman M, Aroyo A, Yavin S, Roth Z. Seasonal effects on gene expression, cleavage timing, and developmental competence of bovine preimplantation embryos. Reproduction. 2010;140:73-82.

[25] Rivera RM, Hansen PJ. Development of cultured bovine embryos after exposure to high temperatures in the physiological range. Reproduction. 2001;121:107-15.

[26] Maxwell WM, Evans G, Hollinshead FK, Bathgate R, De Graaf SP, Eriksson BM, et al. Integration of sperm sexing technology into the ART toolbox. Anim Reprod Sci. 2004;82-83:79-95.

[27] Sartori R, Souza AH, Guenther JN, Caraviello DZ, Geiger LN, Schenk JL, et al. Fertilization rate and embryo quality in superovulated Holstein heifers artificially inseminated with X-sorted or unsorted sperm. Anim Reprod. 2004;1:86-90.

[28] Palma GA, Olivier NS, Neumüller C, Sinowatz F. Effects of sex-sorted spermatozoa on the efficiency of in vitro fertilization and ultrastructure of in vitro produced bovine blastocysts. Anat Histol Embryol. 2008;37:67-73.

[29] Sales JN, Neves KA, Souza AH, Crepaldi GA, Sala RV, Fosado M, et al. Timing of insemination and fertility in dairy and beef cattle receiving timed artificial insemination using sex-sorted sperm. Theriogenology. 2011;76:427-35.

Figure legend

Supplemental Figure 1. Effects of month of insemination on the conception rate after artificial insemination with conventional and sexed semen. The data are represented as differences between the mean conception rate throughout the study period and for each month.

Table 1. Factors affecting the conception rate

Effect	Df	Relative χ^2	$P > \chi^2$
Year	4	6.132	< 0.001
Heifer age	9	3.307	< 0.001
Time of the year	11	10.589	< 0.001
Semen type	1	287.551	< 0.001
service sire	146	3.796	< 0.001
Age × semen type	9	2.357	0.012
Month × semen type	11	3.047	< 0.001

Table 2. Number of AI attempts, percentage of semen type used for AI, and raw conception rate in each year at insemination.

	Conventional			Sexed		
Year	*n*	Percentage	CR[1]	*n*	Percentage	CR[1]
2012	10,343	64.4	55.5	5,730	35.6	42.8
2013	9,257	54.9	55.3	7,598	45.1	46.0
2014	8,110	48.1	56.9	8,762	51.9	47.3
2015	7,931	39.8	58.6	11,978	60.2	48.7
2016	6,216	35.3	59.4	11,397	64.7	49.0
All	41,857	47.9	56.9	45,465	52.1	47.3

[1]CR: conception rate

Table 3. Number of AI attempts, raw conception rate, and odds ratio in each heifer age at insemination with conventional and sexed semen.

	Conventional				Sexed			
Age	*n*	CR[1]	OR[2]	99% CI[3]	*n*	CR[1]	OR[2]	99% CI[3]
≤ 12	6,039	54.8	1[ref]	-	10,089	46.6	0.79	0.67–0.92
13	8,889	56.3	1.05	0.96–1.14	11,920	48.6	0.85	0.69–1.05
14	10,047	56.7	1.06	0.98–1.16	10,565	46.7	0.78	0.63–0.96
15	6,530	56.9	1.07	0.97–1.17	5,735	47.1	0.80	0.64–0.99
16	3,994	57.3	1.08	0.97–1.21	3,247	47.6	0.82	0.64–1.04
17	2,283	58.1	1.11	0.98–1.27	1,725	46.2	0.78	0.59–1.03
18	1,465	57.9	1.11	0.96–1.30	874	44.4	0.72	0.52–1.00
19	821	58.7	1.15	0.94–1.39	508	49.6	0.90	0.61–1.34
20	596	61.2	1.28	1.02–1.60	302	47.4	0.84	0.52–1.34
≥ 21	1,193	64.6	1.51	1.28–1.80	500	47.8	0.86	0.59–1.25
All	41,857	56.9	-	-	45,465	47.3	-	-

[1]CR: conception rate

[2]OR: odds ratio

[3]CI: confidence interval

[ref]Reference

Table 4. Number of AI attempts, raw conception rate, and odds ratio at each month of insemination with conventional and sexed semen.

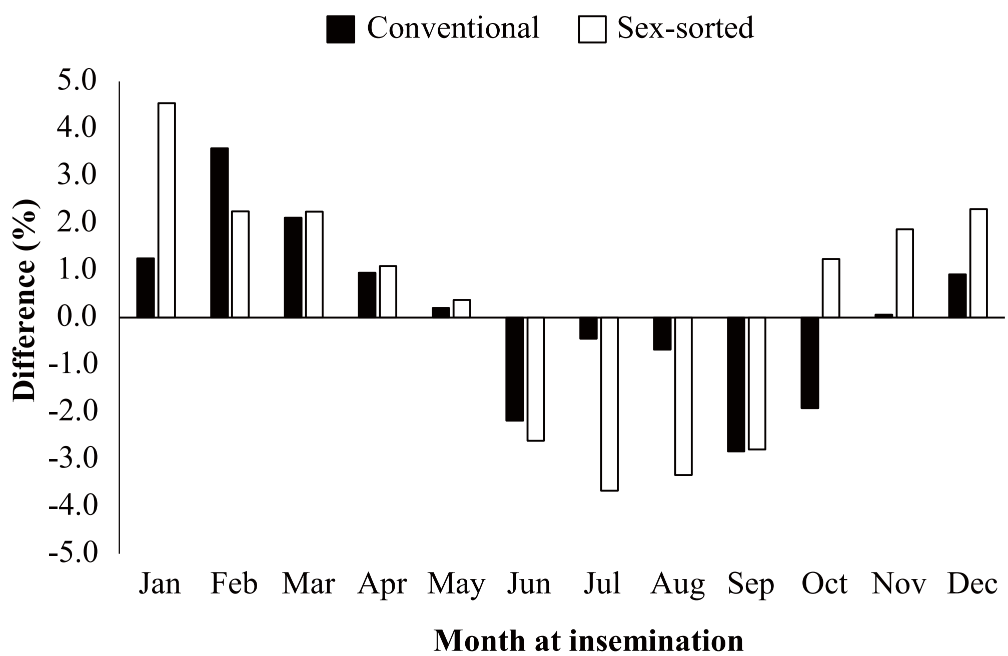
	Conventional				Sexed			
Month	*n*	CR[1]	OR[2]	99% CI[3]	*n*	CR[1]	OR[2]	99% CI[3]
1	3,234	58.1	1[ref]	-	3,033	51.8	0.79	0.67–0.92
2	3,083	60.5	1.11	0.97–1.27	3,010	49.5	0.72	0.55–0.95
3	3,390	59.0	1.04	0.92–1.19	3,200	49.5	0.73	0.55–0.95
4	3,486	57.8	1.01	0.88–1.14	3,460	48.4	0.69	0.53–0.91
5	3,353	57.1	0.97	0.85–1.10	3,672	47.7	0.68	0.51–0.89
6	4,479	54.7	0.89	0.79–1.01	5,045	44.7	0.60	0.46–0.78
7	3,925	56.4	0.95	0.84–1.08	4,523	43.6	0.57	0.44–0.75
8	3,482	56.2	0.94	0.82–1.07	4,070	44.0	0.58	0.44–0.76
9	3,349	54.0	0.86	0.75–0.98	3,841	44.5	0.60	0.46–0.79
10	3,273	55.0	0.89	0.78–1.02	3,633	48.5	0.70	0.53–0.92
11	3,358	56.9	0.97	0.85–1.10	3,989	49.2	0.71	0.54–0.93
12	3,445	57.8	0.99	0.87–1.13	3,989	49.6	0.72	0.55–0.94
All	41,857	56.9	-	-	45,465	47.3	-	-

[1]CR: conception rate

[2]OR: odds ratio

[3]CI: confidence interval

[ref]Reference



Supplemental figure 1.