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Effects of use of conventional and sexed semen on conception rate, calf sex, calf birth weight, and stillbirth in Holstein heifers

Tahir BAYRIL*

Department of Animal Sciences, Faculty of Veterinary Medicine, Dicle University, Diyarbakır, Turkey

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Abstract: Pregnancy rate is low in artificial insemination with sexed semen compared to conventional semen. This situation poses a major problem for dairy farms. The aim of this study was to determine the effects of semen type on conception rate, calf sex and birth weight, stillbirth, pregnancy loss, and postpartum maternal body weight (MBW) in Holstein heifers inseminated with sexed (n = 229), and conventional semen (n = 153). The female and male calf ratios of the heifers inseminated with sexed and conventional semen were found to be 88% to 54.8% and 12% to 45.2%, respectively. There was a significant difference between sperm type and calf sex ($p < 0.001$). While 36.7% of calves between 34 and 36 kg birth weights were born from heifers inseminated with sexed semen, 15.7% were born from heifers inseminated with conventional semen. Moreover, 46% and 17.3% of calves that had birth weights of 36.1–38.1 kg and > 38.2 kg were born from heifers inseminated with sexed semen while 40% and 44.3% were born from heifers inseminated with conventional semen, respectively. A statistically significant relationship was found between semen type and calf birth weights ($p < 0.001$). After calving, there was a significant difference between semen type and MBW after calving ($p < 0.001$). In the sexed and conventional semen groups, the MBW after calving was found to range from 11.3% to 23.5% in the 400–460 kg, 46.7% to 57.4% in the 461–511 kg, and 42% to 19.1% in the 512–587 kg weight categories, respectively. There was no statistically significant difference ($p > 0.05$) between sperm type and mean gestation length and calf birth weight, pregnancy loss, stillbirth rate, and weaning calf mortality rates. There was a strong positive correlation between semen type, sex, and calf birth weight. Furthermore, there was a significant correlation between the MBW, the gestation period and the survival status of the calves. It was determined that there was a minor positive relationship between the insemination season and the gestation period, MBW, and the survival status of the calves. Our results demonstrated that with careful and consistent application of herd management program in dairy cattle farms, the rates of pregnancy obtained with sexed and conventional semen will increase, whilst parameters such as stillbirth and pregnancy loss will decrease.

Key words: Calf sex, conception rate, maternal body weight, sexed semen, stillbirth

1. Introduction

In comparison to conventional mating techniques, reproductive technology advances such as sexed semen, artificial insemination and embryo transfer have led to increased genetic improvement in dairy cattle [1,2]. One of the biggest problems seen in dairy cattle breeding enterprises is the inability to maintain the herd size. Insufficient female breeding animal material worsens this problem. The high rate of female offspring obtained from sexed semen is a development that breeders have been anticipating for a long time. A large proportion of female heifers will contribute to the growth and expansion of a herd, enhancing genetic progress and biosecurity [3]. In 1989, X and Y chromosomes were successfully separated by flow cytometry after much research and many trials [4]. With the help of this sorting method, sexed semen for use in artificial insemination may now be frozen and sold. The breeders' use of this technique to obtain more breeding heifers has made it possible to increase the profitability of the dairy farms. The high genetic level of calves obtained using sexed semen increases yields and genetic progress in dairy herds. [5-7].

One of the major disadvantages of insemination with sexed semen is that it leads to lower pregnancy rates than conventional semen insemination. The pregnancy rate of sexed semen is 75% to 90% of conventional semen [8-10]. The pregnancy rates in Holstein heifers were 27% and 70% with sexed semen, and 34% and 83% with conventional semen [11]. In another study on Holstein heifers, pregnancy rates after estrus follow-up were found to be 59.1% in conventional semen and 48.6% in sexed semen [12]. However, in recent years, sexed sperm manufacturing technology advancements and an increase in semen doses have led to an increase in pregnancy rates. Pregnancy rates

* Correspondence: tbayril@hotmail.com

by years have increased from 35% in 2012 to 67% in 2016 for insemination with sexed sperm [13,14]. In fact, Lenz et al. [14] observed in their study that the pregnancy rate they obtained from sexed semen surpassed conventional semen (66.73% to 65.66%).

Pre- and postnatal calf mortality, as well as the profitability of dairy herds, are influenced by calf birth weight. Male calves having a higher birth weight than female calves increases calf mortality in first-born heifers [15]. In heifers, the use of sexed semen reduces dystocia costs by 20% [16]. In a study conducted with Holstein heifers, it was reported that the stillbirth rates for female calves were 9.2% for sexed semen and 10.5% for conventional semen [11]. Moreover, Borchersen and Peacock (2009) [17] reported stillbirth rates of 10%–14% for female and male calves, respectively, from sexed semen compared to 12%–20% from conventional semen. In cattle breeding, the future of the herd is determined by the female calves that are born. This is more important for dairy cattle farms. Since there is an insufficient number of breeding females to ensure the continuity of the herd, this is a major issue for dairy cattle farms. Lack of breeding females directly affects the genetic progress and productivity of the farm. This study was designed to determine the effects of sexed and conventional semen on pregnancy rate, calf sex and birth weight, pregnancy loss and stillbirth in Holstein heifers.

2. Materials and methods

2.1. Animal management, feeding, and data collection

A private Holstein dairy cattle farm with 2000 heads engaged in dairy cow breeding conducted this investigation from December to May. A total of 385 Holstein heifers, which were born from the farm's own breeding animals and had completed 13 months of age (inseminated age = 15.9 months), reached an average body weight of 350 kg and showed signs of estrus were used for the study. Blood tests for *Brucella*, IBR, BVD, and *Neospora caninum* were performed on all heifers before the trial and only healthy heifers were used in the study. Of these heifers, 230 were inseminated with sexed semen (Holstein bull) (2.5×10^6 sperm/dose) and 155 with conventional semen (Holstein bull) (20×10^6 sperm/dose). The flow cytometric method is used to identify the sperm's sex. In the flow cytometric method, semen is diluted at extremely low concentrations. Sperm are stained using the specific DNA binding dye Hoechst 33342 (40-6-diamino-2-phenylindole). These stained sperms are directed to the device at a pressure of 40 psi and a speed of 60 km/h. Sperm fluoresce according to the amount of dye they absorb. The X chromosome in bull sperm contains about 4% more DNA than the Y chromosome, hence giving it a brighter fluorescence. The difference in the emitted fluorescence is measured by the photomultiplier in the device and analyzed by the computer in the system. Not all sperm can be identified using the photomultiplier. With the help of a vibrator, approximately 70,000–80,000 droplets pass through the nozzle of the device per second. About a third of the droplets carry sperm, while the rest is empty, very few contain two or more sperm. The droplet is electrically charged positively if it contains sperm with X chromosome and negatively if it contains sperm with Y chromosome. As the droplets fall from the flow cytometry nozzle, the positively charged ones are segregated on one side and the negatively charged ones on the other. Dyes in damaged or dead sperm quench Hoechst 33342 fluorescence; therefore, the weakly fluorescent ones are discarded. As a result, at the end of the separation process, the sperm are separated into 3 different tubes [18]. Three heifers were excluded from the study due to health problems, two of them were from the conventional semen group and one from the sexed semen group. The pedometers (DairyPlan C21, GEA Herd Management, WestfaliaSurge, Germany) on the heifers' feet and the shepherd's daily controls were used to identify the signs of estrus. Pedometers record the number of steps taken per unit time as an indicator of increased walking activity. Activity data is then scanned and downloaded to the computer software via an antenna. This information is stored and calculated in the herd management software. The software calculates the average values for each cow's stepping and lying activity. If the cow's activity deviates from its average activity, the system alerts the operator [19]. The estrus index in the estrus detection system established in the enterprise ranges from 0 to 200. The first hour when the activity value was above 70 was considered the onset of estrus, and the first hour when it was below 70 again was considered the end of estrus. The peak activation index during the estrus period was acknowledged as the maximum estrus index. Heifers' maximum activation indices of 70–110 during estrus was considered low, and >110 was considered high. A veterinarian artificially inseminated (intrauterine) heifers that showed signs of estrus and had a functional follicle on one of their ovaries in rectal and ultrasound examination and did not have any abnormal discharge. Artificial insemination was performed by evaluating the activity index and ultrasound findings. Artificial insemination was performed 8–10 h after the detection of the dominant follicle with a high activation index. Only one dose per heifer was used due to the high cost of sexed semen. As a result, the first insemination parameters of heifers inseminated with sexed and conventional semen were assessed. The semen used in both groups was obtained from the same breed of bull. The body weights of the inseminated and calving heifers were recorded using an electronic scale (Taxatron, WestfaliaSurge, Germany) connected to the central computer in the parlor. Thirty-two to fourty-five days after insemination, the animals were rectally examined for pregnancy using an ultrasound with a 5–7.5-MHz linear probe (Agroscan L, MWM Medical, İstanbul, Türkiye) and the pregnant heifers were identified. Pregnancy was confirmed by the observation of the fetal heartbeat. Pregnant animals were reexamined using ultrasound at day 60 of pregnancy, and nonpregnant animals were recorded as pregnancy loss. Insemination date, gestational status, calving date, pregnancy loss, stillbirths, deaths at 0–2 months (weaning age), calf sex, and calf birth weights are all recorded as a part of the herd management program (DairyPlan C21, WestfaliaSurge, Germany). All data regarding the heifers and calves were printed out from the herd management program of the enterprise for analysis. Heifers were housed in free system and semi-open shelters. Heifers were fed twice a day, once in the morning and once in the evening. A total mixed ration (TMR) consisting of 6 kg corn silage, 2 kg of dry alfalfa hay, 1 kg vetch + triticale hay, and 2 kg heifer concentrated feed (HP: 16%; ME: 2600 kcal/kg) were given to them at the age of 15–18 months. During the 19–22-month period, 8 kg of corn silage, 2 kg of dry alfalfa hay, 1 kg of vetch + triticale hay, and 2 kg of heifer concentrated feed were given. The last 2 months of pregnancy were split into two periods. Heifers were fed 10 kg of corn silage, 2 kg of vetch + triticale hay, and 3 kg of heifer concentrated feed throughout the first month. For a successful transition into the lactation period, in the last period of pregnancy and 15 days before calving, 12 kg of corn silage, 1 kg of dry alfalfa hay, 2 kg of vetch + triticale hay, 2 kg of heifer concentrated feed, and 4 kg of early lactation feed (HP: 18%; ME: 2850 kcal/kg) were given.

2.2. Statistical analysis

Descriptive statistics for continuous variables were expressed as mean, standard deviation, minimum and maximum values, while numbers and percentages were used for categorical variables. Chi-squared test and multiple correspondence analysis were used to determine the relationship between categorical variables. The statistical significance level was considered 5% and SPSS (ver: 21) statistical package program was used for all statistical computations.

3. Results

The effect of semen type (Holstein bulls) on calf sex is shown in Table 1. Male calf rates in the sexed and conventional semen groups ranged from 12% to 45.2%, and female calf rates ranged from 88.0% to 54.8%, respectively. A significant difference was found between semen type and calf sex ($p < 0.001$).

In the current study, the relationship between Holstein calf birth weight and semen type was evaluated. In the sexed and conventional semen groups, it was shown to range from 36.7% to 15.7% in calves at a birth weight of 34–36 kg, 46% to 40% at a birth weight of 36.1–38.1 kg, and 17.3% to 44.3% for a birth weight of >38.2 kg, respectively. Semen type and birth weights of calves were shown to be statistically correlated ($p < 0.001$) (Table 2).

The maternal body weight (MBW) means after calving in the sexed and conventional semen groups ranged from 11.3% to 23.5% in the 400–460 kg, 46.7% to 57.4% in the 461–511 kg, and 42% to 19.1% in the 512–587 kg, respectively. A significant relationship was found between

Table 1. The effect of semen type on calf sex.

Sex		Semen type		Total
		Sexed semen	Conventional semen	
Male	Count	18	52	70
	% within sex	25.7	74.3	100
	% within semen type	12.0[a]	45.2[b]	26.4
	% of total	6.8	19.6	26.4
Female	Count	132	63	195
	% within sex	67.7	32.3	100
	% within semen type	88.0[a]	54.8[b]	73.6
	% of total	49.8	23.8	73.6
Total	Count	150	115	265
	% within sex	56.6	43.4	100
	% within semen type	100	100	100
	% of total	56.6	43.4	100

Chi-square = 36.952; p = 0.001

Table 2. Effect of semen type on calf birth weight.

Sexed semen Conventional semen			Semen type		Total
Birth weight of calf	34–36 kg	Count	55	18	73
		% within birth weight of calf	75.3	24.7	100
		% within semen type	36.7[a]	15.7[b]	27.5
		% of total	20.8	6.8	27.5
	36.1–38.1 kg	Count	69	46	115
		% within birth weight of calf	60.0	40.0	100
		% within semen type	46.0[a]	40.0[b]	43.4
		% of total	26.0	17.4	43.4
	> 38.2 kg	Count	26	51	77
		% within birth weight of calf	33.8	66.2	100
		% within semen type	17.3[a]	44.3[b]	29.1
		% of total	9.8	19.2	29.1
Total % within birth weight of calf % within semen type % of total		Count	150	115	265
		56.6	43.4	100	
		100	100	100	
		56.6	43.4	100	

Chi-square = 27.324; p = 0.001

the semen type and maternal body weight values of the calves after calving ($p < 0.001$) (Table 3).

Pregnancy rates were found as 70.7% and 77.8% in sexed and conventional semen groups, respectively. Although the pregnancy rate of heifers inseminated with conventional semen showed a positive increase compared to those inseminated with sexed semen, there was no statistically significant difference between semen type and pregnancy rate ($p > 0.05$). The mean durations of pregnancy were 276.1 ± 16.1 and 276.9 ± 15.6, respectively. The rates of pregnancy loss ranged from 7.4% to 3.4%, stillbirth rate 6.0% to 5.2%, mean calf birth weight 36.6 ± 3.8 to 37.7 ± 3.8 kg, and weaning mortality rate in calves 11.3% to 5.2%, respectively. There was no statistical difference between sperm type and these parameters ($p > 0.05$). There was a strong positive correlation between semen type and sex and calf birth weight. Additionally, a strong correlation between MBW, and calf survival status after calving was found (Table 4; Figure 1).

The rates of male and female calves were observed to be 22.9% to 14.4% in cows with a body weight of 400–460 kg, 52.9% to 50.8% in cows with a body weight of 461–511 kg, and 24.3% and 34.9% in cows with a body weight of 512–587 kg, respectively. The rates of male and female calves obtained from animals inseminated in the spring season were 44.3% and 55.7%, while the rates of male and female calves obtained from animals inseminated in the winter season were 54.9% and 45.1%, respectively. The rates of male and female calves by gestation period in heifers were found to be 12.3% to 11.3% on days 260–270, 70% to 74.9% on days 271–281, and 17.1% to 13.8% on days 282–295, respectively. The male and female rates of calves with a birth weight of 34–36, 36.1–38.1, and >38.2 kg were calculated as 6.8% to 93.2%, 6.8% to 93.2%, and 58.4% to 41.6%, respectively. The relationship between calf sex and birth weight was found to be statistically significant ($p < 0.001$). A positive correlation was observed between categories of sexed sperm, calf weight 36.1–38.1 kg, and postnatal live weight 512–587 kg. The categories of insemination season spring, calf weight (34–36 kg), calf sex (female), gestation period 260–270 days, and calf death (at 0–2 months) are in the lower right area, which is positive in the first dimension and negative in the second dimension. There were conventional sperm categories of sperm type and 400–460 kg and 461–511 kg of MBW in the lower left quadrant, which is the negative region for both dimensions. Heifers inseminated with conventional semen should expect a gestation period of 282–295 days and a postpartum body weight in the range of 400–511 kg. The gestation period of the heifers inseminated in winter is expected to be 271–281 days, and the birth weight of the calves and stillbirths will both be 38.2 kg stillbirths (Table 5; Figure 2).

Table 3. The effect of semen type on MBW.

Sexed semen Conventional semen			Semen type		Total
MBW	400– 460 kg	Count	17	27	44
		% within MBW	38.6	61.4	100
		% within semen type	11.3[a]	23.5[b]	16.6
		% of total	6.4	10.2	16.6
	461–511 kg	Count	70	66	136
		% within MBW	51.5	48.5	100
		% within semen type	46.7[a]	57.4[b]	51.3
		% of total	26.4	24.9	51.3
	512–587 kg	Count	63	22	85
		% within MBW	74.1	25.9	100
		% within semen type	42.0[a]	19.1[b]	32.1
		% of total	23.8	8.3	32.1
Total % within MBW % within semen type % of total		Count	150	115	265
		56.6	43.4	100	
		100	100	100	
		56.6	43.4	100	

Chi-square = 17.856; p = 0.001

MBW: Maternal body weight after calving.

Table 4. The effect of semen type on calf parameters and gestation length.

Item	Sexed semen (n)	Conventional semen (n)	p-value
Conception rate (%)	70.7 (162/229)	77.8 (119/153)	= 0.127
Gestation length (days), mean ± SD	276.1 ± 16.1 (150)	276.9 ± 15.6 (115)	= 0.720
Pregnancy loss (%)	7.4 (12/162)	3.4 (4/119)	= 0.148
Stillbirth (%)	6.0 (9/150)	5.2 (6/115)	= 0.785
Birth weight of calf (kg), mean ± SD	36.6 ± 3.8 (150)	37.7 ± 3.8 (115)	= 0.128
Calf death (at 0–2 months)* (%)	11.3 (16/141)	5.5 (6/109)	= 0.106

*Calf weaning mortality rate

Stillbirth: It was defined for calves which were stillborn after day 260 of gestation and died within 48 h of calving.

Pregnancy loss: It was defined as the ratio of heifers that were pregnant on the 32nd– 45th day of insemination to the heifers that were no longer pregnant at the 60th day of gestation.

4. Discussion

Due to its high cost and lower insemination success than normal semen, sexed semen is used once or twice for insemination of heifers and cows. Heifers and cows are inseminated with the less costly conventional semen [20]. In the study, due to the high cost of sexed semen, it was only used in heifers. Male and female rates of calves born from sexed semen were 88% and 12%, respectively. The sex distribution of the calves born from sexed semen is similar to those in the literature. Diers et al. [21] reported female semen rate as 87%, Djedović et al. [22], DeJarnette et al. [11], and Healy et al. [23] reported it as 85.1%, 89%, and 86%, respectively. These rates were 54.8% and 45.2% in conventional semen. The ratio of male and female in conventional semen was slightly different from expectations. Generally, the ratio of male and female calves

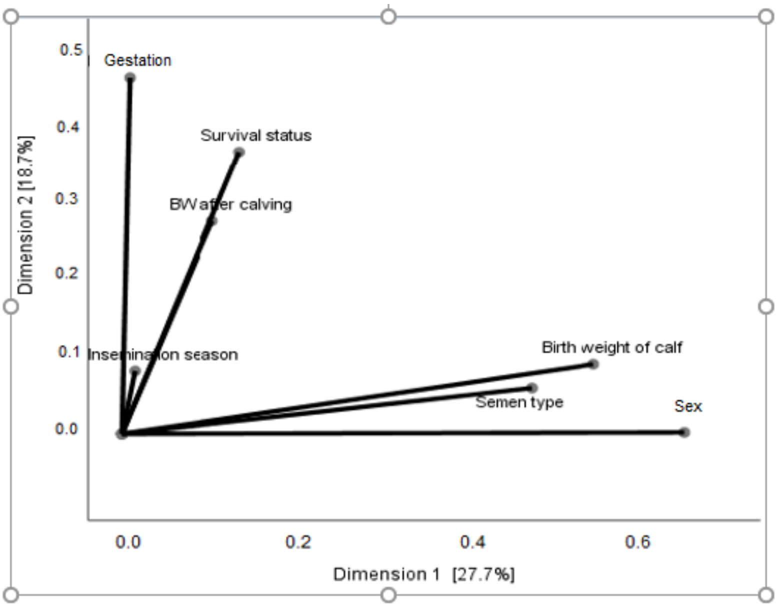

Figure 1. Configuration for the relationships among traits in two-dimensional map.

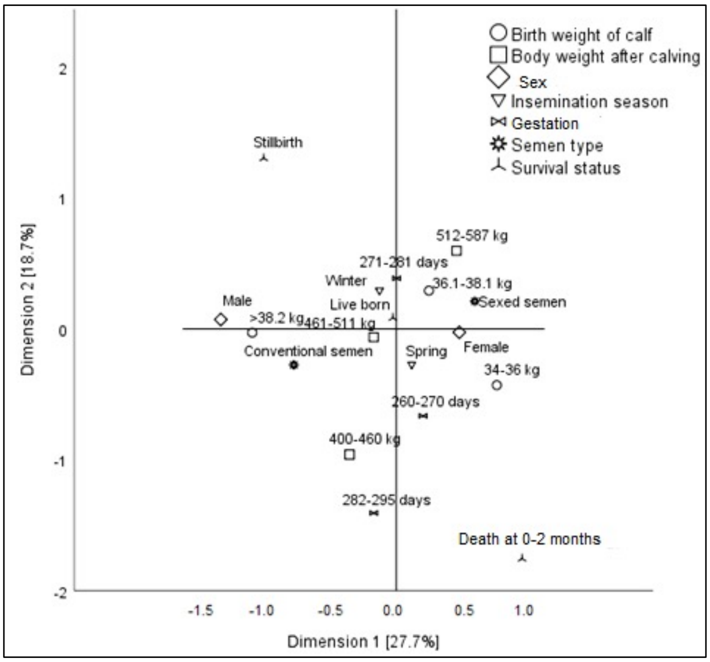

Figure 2. Configuration for the relationships among categories of the traits in two-dimensional map.

is approximately equal to each other. It has been 50% to 52% in many studies [11,24]. The closest ratio to the presented study is Diers et al. [21] and Norman et al. [15] (52.7% to 47.3% and 51.5% to 48.5%). These differences may be due to different environmental conditions or artificial insemination methods.

In cattle breeding, calf birth weight is very important. There are many factors which can affect calf birth weights. These are genetic and nongenetic factors. These factors include calf sex, maternal age, and body weight, gestational age, number of calves, and birth season [25,26]. In our study, the effects of semen type and calf sex on calf birth weights were significant, and a positive correlation was observed between them (Figure 1). Calves born from heifers inseminated with sexed semen had low birth weights, whereas calves born from heifers inseminated

Table 5. Effect of calf sex on MBW, insemination season, gestation length, and CBW.

Male Female			Sex		Total
MBW	400–460 kg	N	16	28	44
		%	22.9	14.4	16.6
	461–511 kg	N	37	99	136
		%	52.9	50.8	51.3
	512–587 kg	N	17	68	85
		%	24.3	34.9	32.1
Chi-square = 4.084; p = 0.130					
Insemination season	Spring	N	31	107	138
		%	44.3	54.9	52.1
	Winter	N	39	88	127
		%	55.7	45.1	47.9
Chi-square = 2.313; p = 0.128					
Gestation length	260–270 days	N	9	22	31
		%	12.9	11.3	11.7
	271–281 days	N	49	146	195
		%	70.0	74.9	73.6
	282–295 days	N	12	27	39
		%	17.1	13.8	14.7
Chi-square = 0.656; p = 0.720					
Birth weight of calf	34–36 kg	N	5	68	73
		%	7.1[a]	34.9[b]	27.5
	36.1–38.1 kg	N	20	95	115
		%	28.6[a]	48.7[b]	43.4
	> 38.2 kg	N	45	32	77
		%	64.3[a]	16.4[b]	29.1
Chi-square = 59.827; p = 0.001					

with conventional semen had high birth weights. This is due to the higher number of females born using sexed semen. Additionally, a positive correlation was observed between calves with a birth weight of 36.1–38.1 kg and cows with a postpartum body weight of 512–587 kg. Using sexed sperm, calf birth weight is expected to be within the range of 36.1–38.1 kg and postpartum MBW within the range of 512–587 kg. There is also a positive correlation observed between 34–36 kg calves, insemination season, female calves, and gestation period. If the insemination season is spring, gestation period and birth weight of female calves are expected to be within the range of 260–270 days and 34–36 kg, respectively (Figure 2). Similarly, some researchers reported that the effect of semen type and insemination season on calf birth weight is important [22,24].

The transition from the dry period to lactation is a crucial and difficult period. Although the amount of milk increases rapidly, feed consumption does not occur at the same rate. This energy demand results in the mobilization of fat and muscle tissues, as the cow cannot consume enough dry matter to meet the energy requirements from rapid milk production [27]. Body weight losses during birth and early lactation significantly affect reproductive performance, according to research [28,29]. In the study, MBW and pregnancy rates in heifers inseminated with sexed and conventional semen were 400–460, 461–511, and 512–587 kg, and 11.3% to 23.5%, 46.7% to 57.4%, and 42 to 19.1%, respectively, after calving. This data indicates that heifers that become pregnant with sexed semen have a higher MBW after birth. A high rate of offspring born from sexed semen is female calves. It is known that female calves have lower birth weights compared to males. The lower body weight of the calf in the dam's uterus in the last period of pregnancy may reduce the pressure on the digestive system [15]. This may have contributed to the body weight gain by making the heifer consume more feed.

The higher pregnancy rates of heifers and the cost of sexed semen cause heifers to be used more. Despite this, the pregnancy rate of sexed semen is lower than conventional semen [15,30]. In the flow cytometry method, the sperm functions are altered and capacitation begins by changing the sperm functions in the separation process of the semen, and the life span in the female genital tract is shortened and the fertilization ability is reduced, thus resulting in a decrease in the pregnancy rate of the sexed sperm [30–32]. The pregnancy rates of sexed and conventional semen were calculated as 70.7% and 77.8%, respectively, in this study. The rate that came closest to our study (66.73% to 65.66%) was reported by Lenz et al. [14]. The difference between the current study and other reported ones is that the pregnancy rate of sexed semen is higher than that of conventional semen. This is because of the high dose of sexed semen used (4 million/straw). The low pregnancy rate in sexed semen may be due to the factors stated above. The pregnancy rates of the two groups in our study were greater than many reported studies. In a study conducted in lactating cows, pregnancy rates were found to be between 49.58% and 67.1% [33]. In a study conducted for the comparison of different sperm doses, the pregnancy rates of sexed and conventional semen were reported to be 46% to 61% [34] and 54% to 60% [35], respectively. In fixed-time insemination with sexed semen, the pregnancy rate in primiparous cows has been reported to be 50% [36]. Oikawa et al. [13] reported pregnancy rates of 64.4% to 35.6% and 64.7% to 35.3% in 2012 and 2016, respectively, in a thorough investigation comparing sexed and conventional semen. It is evident from this data that the pregnancy rate has increased considerably in heifers that were inseminated with sexed semen. Researchers attribute this to the development of a novel method by a Japanese company to increase the fertilization capacity of sexed semen. There are some reasons for the higher pregnancy rates in the presented study than the other reported studies. The first is that both herders and activation meter equipment can identify estrus in cows. The second is that heifer insemination using ultrasound to determine the ideal insemination timing by a specialist veterinarian. The efficiency of the herd management program used in the farm, the use of both activity meters and ultrasound in estrus detection, and the strict application of disease control may have helped increase the pregnancy rate. Third, the published investigations used various artificial insemination methods and synchronization programs. And fourth, the warmer insemination season (December–May) may have increased the pregnancy rate.

The gestation period is a species-specific feature. Mean gestation length were 276.1 and 276.9 days, respectively. There was no statistically significant difference between the gestational periods. According to studies, the average gestation period varies between 273 and 282 days [15]. Similar results have been reported by other researchers as well [22,33,37]. Pregnancy loss rates were found to be 7.4% and 3.4% in the groups, respectively, and pregnancy loss was observed to be higher in sexed semen. However, there was no significance found between the groups. Lauber et al. [36] used two different insemination protocols, and estimated the pregnancy losses in sexed semen as 5% and 6%, respectively. This rate was slightly lower than the presented study. In another study in which three different insemination protocols were applied using sexed and conventional semen, the rates were reported as 9.5% to 8.6%, 12.0% to 25%, and 4.6% to 6.7%, respectively [5], and these findings are considerably greater than our values. The physical and chemical methods used to separate the spermatozoa may have caused DNA damage, which may have contributed to higher pregnancy losses in heifers inseminated with sexed semen [38]. Stillbirth rates were similar in heifers inseminated with sexed and conventional semen (6% vs 5.2%). Our findings were lower than the findings of many researchers. These rates in sexed and conventional semen were reported as 8.4% to 7.2%, 7.54% to 6.19%, 13.3% to 11.6%, and 11.3% to 10.4%, respectively [22,23,39,40]. The fact that the findings of our study were lower than those previously reported can be explained by the blood tests (*Brusella*, IBR, BVD, *Neospora caninum*) of the heifers to be included in the study and the inclusion of the negative ones in the trial.

5. Conclusion

As a result, in order to increase pregnancy rates in sexed semen, the effective use of herd management programs, the combined use of activity meter and ultrasound for estrus detection, and the eradication of the disease will all positively affect the reproductive parameters.

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References

1. Boneya G. Sexed semen and major factors affecting its conception rate in dairy cattle. International Journal of Advance Research Biological Sciences 2021; 8 (1): 99-107. http://dx.doi.org/10.22192/ijarbs.2021.08.01.012

2. Vishwanath R. Artificial insemination: the state of the art. Theriogenology 2003; 59: 571-584. https://doi.org/10.1016/S0093-691X(02)01241-4

3 Weigel KA. Exploring the impact of sexed semen in dairy production systems. Journal of Dairy Science 2004; 87 (E. Suppl.): 120-130. https://doi.org/10.3168/jds.S0022-0302(04)70067-3

4. Johnson LA, Flook JP, Hawk HW. Sex preselection in rabbits: Live births from X and Y sperm separated by DNA and cell sorting. *Biology* of *Reproduction* 1989; 41: 199-203. https://doi.org/10.1095/biolreprod41.2.199

5. Karakaya - Bilen E, Yilmazbas - Mecitoglu G, Keskin A, Guner B, Serim E et al. Fertility of lactating dairy cows inseminated with sex - sorted or conventional semen after Ovsynch, Presynch - Ovsynch and Double - Ovsynch protocols. Reproduction in Domestic Animals 2019; 54 (2): 309-316. https://doi.org/10.1111/rda.13363

6. Holden SA, Butler ST. Review: Applications and benefits of sexed semen in dairy and beef herds. Animal 2018; 12: 97-103. https://doi.org/10.1017/S1751731118000721

7. Taylor JF, Schnabel RD, Sutovsky P. Review: Genomics of bull fertility. Animal 2018; 12: 172-183. https://doi.org/10.1017/S1751731118000599

8. Maicas C, Holden SA, Drake E, Cromie AR, Lonergan P et al. Fertility of frozen sex-sorted sperm at 4 × 106 sperm per dose in lactating dairy cows in seasonal - calving pasturebased herds. Journal of Dairy Science 2020; 103: 929-939. https://doi.org/10.3168/ jds.2019-17131

9. Perry GA, Walker JA, Rich JJJ, Northrop EJ, Perkins SD et al. Influence of sexcel (gender ablation technology) gender - ablated semen in fixed - time artificial insemination of beef cows and heifers. Theriogenology 2020; 146: 140-144. https://doi.org/10.1016/j.theriogenology .2019.11.030

10. Seidel GE. Update on sexed semen technology in cattle. Animal 2014; 8 (s1): 160-164. https://doi.org/10.1017/S1751731114000202

11. DeJarnette JM, Nebel RL, Marshall CE. Evaluating the success of sex - sorted semen in US dairy herds from on farm records. Theriogenology 2009; 71 (1): 49-58. https://doi.org/10.1016/j.theriogenology.2008.09.042

12. Miura R, Izumi T. Relationship of the conception rate and the side (left or right) of preovulatory follicle location at artificial insemination in dairy heifers. Animal Science Journal 2018; 89: 328-331. https://doi.org/10.1111/asj.12949

13. Oikawa K, Yamazaki T, Yamaguchi S, Abe H, Bai H et al. Effects of use of conventional and sexed semen on the conception rate in heifers: A comparison study. Theriogenology 2019; 135: 33-37. https://doi.org/10.1016/j.theriogenology.2019.06.012

14. Lenz RW, Gonzalez - Marin C, Gilligan TB, DeJarnette JM, Utt MD et al. 190 SexedULTRA™, A new method of processing sex - sorted bovine sperm improves conception rates. Reproduction, Fertility and Development 2016; **29:** 203-204. https://doi.org/10.1071/RDv29n1Ab190

15. 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. Journal of Dairy Science 2010; 93 (8): 3880-3890. https://doi.org/10.3168/jds.2009-2781

16. Seidel GE. Economics of selecting for sex: the most important genetic trait. Theriogenology 2003; 59: 585 - 598. https://doi.org/10.1016/S0093-691X(02)01242-6

17. Borchersen S, Peacock M. Danish A.I. field data with sexed semen. Theriogenology 2009; 71: 59 - 63. https://doi.org/10.1016/j.theriogenology.2008.09.026

18. Seidel GE () Overview of sexing sperm. Theriogenology 2007; 68: 443-446. https://doi.org/10.1016/j.theriogenology.2007.04.005

19. Calderón, L. 2002. Reproductive efficiency of a dairy herd after introduction of a pedometry-based estrous detection system. M.Sc. Thesis, University of Puerto Rico, Mayaguez Campus, Puerto Rico. Pp 37.

20. Schenk JL, Cran DG, Everett RW, Seidel GE. Pregnancy rates in heifers and cows with cryopreserved sexed sperm: Effects of sperm numbers per inseminate, sorting pressure and sperm storage before sorting. Theriogenology 2009; 71 (5): 717-728. https://doi.org/10.1016/j.theriogenology.2008.08.016.

21. Diers S, Heise J, Krebs T, Groenewold J, Tetens J. Effect of sexed semen on different production and functional traits in German Holsteins. Veterinary and Animal Science 2020; 9: 100-101. https://doi.org/10.1016/j.vas.2020.100101

22. Djedović R, Bogdanović V, Stanojević D, Nemes Z, Gáspárdy A et al. Involuntary reduction in vigour of calves born from sexed semen. *Acta Veterinaria Hungarica* 2016; 64 (2): 229-38. https://doi.org/10.1556/004.2016.023

23. Healy AA, House JK, Thomson PC. Artificial insemination field data on the use of sexed and conventional semen in nulliparous holstein heifers. Journal of Dairy Science 2013; 96 (3): 1905-1914. https://doi.org/10.3168/jds.2012-5465

24. Tubman LM, Brink Z, Suh TK, Seidel GE. Characteristics of calves produced with sperm sexed by flow cytometry cell sorting. Journal of Animal Science 2004; 82: 1029-1036. https://doi.org/10.2527/2004.8241029x

25. Cundiff LV, MacNeil MD, Gregory KE, Koch RM. Between and withinbreed genetic analysis of calving traits and survival to weaning in beef cattle. Journal of Animal Science 1986; 63: 27-33. https://doi.org/10.2527/jas1986.63127x

26. Nelsen TC, Short RE, Urick JJ, Reynolds WL. Genetic variance components of birth weight in a herd of unselected cattle. Journal of Animal Science 1984; 59: 1459-1466. https://doi.org/10.2527/jas1984.5961459x

27. Bell AW, Bauman DE. Adaptations of glucose metabolism during pregnancy and lactation. *Journal* of *Mammary Gland Biology* and Neoplasia 1997; 2: 265-278. https://doi.org/10.1023/A:1026336505343

28. Buckley F, O'Sullivant K, Mee JF, Evans RD, Dillon P. Relationship among milk yield, body condition, cow weight, and reproduction in spring - calved Holstein - Friesians. Journal of Dairy Science 2003; 86: 2308-2319. https://doi.org/10.3168/jds.S0022-0302(03)73823-5

29. Senatore EM, Butler WR, Oltenacu PA. Relationships between energy balance and post - partum ovarian activity and fertility in first lactation dairy cows. Animal Science 1996; 62: 17-23. https://doi.org/10.1017/S1357729800014260

30. Mallory DA, Lock SL, Woods DC, Poock SE, Patterson DJ. Hot topic: Comparison of sex - sorted and conventional semen within a fixed - time artificial insemination protocol designed for dairy heifers. Journal of Dairy Science 2013; 96: 854-856. https://doi.org/10.3168/jds.2012-5850

31. Kurykin J, Jalakas M, Kaart T, Jaakma Ü. Efficiency of insemination with sexed semen at spontaneous estrus and synchronization of ovulation in lactating holstein cows. Veterinarija ir Zootechnika 2017; 75: 30-35.

32. Schenk JL, Cran DG, Everett RW Jr Seidel GE. Pregnancy rates in heifers and cows with cryopreserved sexed sperm: Effects of sperm numbers per inseminate, sorting pressure and sperm storage before sorting. Theriogenology 2009; 71: 717-728. https://doi.org/10.1016/j.theriogenology.2008.08.016

33. Zargaran A, Amin Afshar M, Joezy - Shekalgorabi S, Azizi J, Chamani M. Reproductive performance of holstein heifers inseminated with sex sorted semen in various herd sizes. *Iranian Journal of Applied Animal Science* 2021; 11 (2): 249-259.

34. DeJarnette JM, McCleary CR, Leach MA, Moreno JF, Nebel RL et al. Effects of 2.1 and 3.5x10(6) sex - sorted sperm dosages on conception rates of Holstein cows and heifers. Journal of Dairy Science 2010; 93 (9): 4079-85. https://doi.org/10.3168/jds.2010-3181

35. Walsh DP, Fahey AG, Mulligan FJ, Wallace M. Effects of herd fertility on the economics of sexed semen in a high - producing, pasture - based dairy production system. Journal of Dairy Science 2021; 104 (3): 3181-3196. https://doi.org/10.3168/jds.2020-18676

36. Lauber MR, McMullen B, Parrish JJ, Fricke PM. Short communication: Effect of timing of induction of ovulation relative to timed artificial insemination using sexed semen on pregnancy outcomes in primiparous Holstein cows. Journal of Dairy Science 2020; 103 (11): 10856-10861. https://doi.org/10.3168/jds.2020-18836

37. Chebel RC, Guagnini FS, Santos JEP, Fetrow JP, Lima JR. Sex - sorted semen for dairy heifers: Effects on reproductive and lactational performances. Journal of Dairy Science 2010; 93 (6): 2496-2507. https://doi.org/10.3168/jds.2009-2858

38. Suh TK, Schenk JL, Seidel GE. High pressure flow cytometric sorting damages sperm. Theriogenology 2005; 64 (5): 1035-1048. https://doi.org/10.1016/j.theriogenology.2005.02.002

39. Jabarzareh A, Sadeghi-Sefidmazgi A, Ghorbani G, Cabrera V. Economic evaluation of sexed semen use in Iranian dairy farms according to field data. Reproduction in Domestic Animals 2018; 53 (6): 1271-1278. https://doi.org/10.1111/rda.13247

40. Norman HD, Wright JR, Kuhn MT, Hubbard SM, Cole JB. Genetic and environmental factors that affect gestation length. Journal of Dairy Science 2007; 90 (Supplement 1): 264. https://doi.org/10.3168/jds.2007-0982