SEX-SORTED SEMEN: EFFICIENCY OF INSEMINATION AND OPPORTUNITIES TO INCREASE OUTCOME OF PREGNANCIES IN DAIRY AND BEEF CATTLE. A REVIEW

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Contents. Insemination with semen, separated by flow cytometry into fractions containing X- or Y-chromosome-bearing populations of sperm, allows one to skew the sex ratio of offspring up to 90% of the desired sex. The disadvantage of using sexed semen is a small insemination dose of functionally impaired sperm, exacerbated by damage during sorting and the result is reduced fertilising potential. With the commercial introduction of sexed semen, its use has been recommended for the insemination of heifers due to the higher fertility potential compared to lactating cows. The benefit and rate of genetic gain would be greater through obtaining additionally high-value offspring from superior cows using the sexed semen of elite bulls. Studies that have aimed to increase the efficiency of insemination with sexed semen have focused on the use of timed insemination programmes. This overview represents data from studies on sexed semen, factors related to pregnancy rates and opportunities favouring an increase of pregnancies in dairy and beef cattle breeds.

Keywords: cattle, sex-sorted semen, insemination, effects, timed programmes

Introduction. The flow cytometrically sex-sorted bovine semen, containing predominantly X- or Y-chromosome-bearing populations of sperm, providing up to 90% of offspring of the desired sex, has been increasingly used in livestock operations worldwide. The impact and profitability of sexed semen use in the dairy and beef industries have been analysed and extensively discussed (Hohenboken 1999; Seidel 2003; Weigel 2004; Abdel-Azim, Schnell 2007; De Vries et al. 2008; Hossein-Zadeh et al. 2010; Ettema et al. 2011; Heikkilä, Peippo 2012). The disadvantage of using sexed semen is a small insemination dose of functionally impaired sperm, exacerbated by damage during sorting and the result is reduced fertilising potential (Seidel et al. 1999; Seidel 2003).

The production rate of the current flow-cytometric technology is limited, as only about 30% of the sperm of a desired sex can be sorted from ejaculate. The limited output and expense have made the extended use of sexed semen economically profitable with the use of a 2.1 x 10^6 sperm per insemination dose (Seidel 2003; Maxwell et al. 2004). Reduction by a high dilution of semen in concentration of protective seminal lipids and proteins, maintaining membrane stability, may lead to the great non-linearity and immotility of sperm (Maxwell et al. 2004). The damage of chromatin stability is associated with the exposure of sperm to pressure, ultraviolet radiation and Hoechst/laser interaction (Boe-Hansen et al. 2005; Garner, 2006; Gosálvez et al. 2011). Capacitation and the acrosome reaction appeared to be accelerated during sorting and freezing-thawing, reducing the functional lifespan of sperm (Moće et al. 2006). The damage of membranes by oxidative stress and a decrease in the motility of frozen-thawed sorted sperm have been found to occur to a greater extent due to their higher susceptibility to cryopreservation stressors compared with non-sorted sperm (Garner 2006).

With the commercial introduction of sexed semen, its use has been recommended for the insemination of heifers due to the higher fertility potential compared to lactating cows. The benefit and the rate of genetic gain would be greater through obtaining replacement heifers from genetically superior cows using the sexed semen of elite bulls (Hohenboken 1999; Seidel 2003; De Vries et al. 2008). Currently, there is increasing interest in the dairy and beef industries in accelerating genetic gain – producing more high-value offspring from the best cows – to offset low fertility and the high culling rate in dairy farms and to increase the economically favourable proportion of mail calves in beef herds (Sales et al. 2011). The primary factors, limiting reproductive performance in dairy and beef cattle herds, are a delay in the resumption of cycling in cows after calving, weak estrous expression and the low detection rates of estrus. To facilitate the use of sexed semen, the need exists for reproductive strategies, optimizing the detection of estrus and the timing of insemination (Sá Filho et al. 2010). Incorporating into reproductive management programmes, allowing the control of ovulation and timed insemination may be the optimum scenario for the use of sexed semen in cattle breeding (DeJarnette et al. 2010a).

This review represents data from studies on sexed semen, factors related to pregnancy rates and opportunities favouring an increase of pregnancies in heifers and lactating cows of dairy and beef breeds.

Efficiency of sexed semen use and factors influencing pregnancy rate

The efficiency of insemination with sexed semen has been studied in heifers and lactating cows of dairy and beef breeds and has remained an object of research to date. Among studies on heifers (Table 1), reported pregnancy rates vary between 31-60% and constitute 54-89% of the rates obtained by using unsexed semen. In studies on cows (Table 2), sexed semen use resulted in extremely low pregnancy rates of 21-25%, especially in lactating dairy cows, or 45-64% of the rates obtained by using unsexed semen. In cows of beef breeds, pregnancy rates (41.8-57%) appear to be comparable with the rates reported for heifers of dairy (31.6-60.2%) and beef (38.8-53%) breeds.

Table 1. **Efficiency of sexed semen use in dairy and beef heifers in different geographic regions**

Country	Breeds	Type of semen		References
		Sexed	Unsexed	
USA	Holstein, Angus	43%	62%	Seidel et al. 1999
Switzerland	Brown Swiss, Red Holstein	33.9%	59.3%	Bodmer et al. 2005
Estonia	Holstein	43%	68%	Kurykin et al. 2007
Italy	Holstein-Friesian	51.5%	-	Cerchiaro et al. 2007
USA	Holstein	43%	62%	Seidel, Schenk 2008
USA	Red Angus	53%	67%	Seidel, Schenk 2008
USA	Black Angus	47%	72%	Seidel, Schenk 2008
Denmark	Holstein	49.3%	61.9%	Borchersen, Peacock 2009
Denmark	Jersey	46.6%	53.9%	Borchersen, Peacock 2009
Denmark	Danish Red	60.2%	65.4%	Borchersen, Peacock 2009
Argentina	Holstein	34.3%	-	Brogliatti et al. 2009
USA	Holstein	43.9%	60.7%	DeJarnette et al. 2010a
USA	Holstein	39%	56%	Norman et al. 2010
USA	Holstein	38.2%	51.9%	Chebel et al. 2010
China	Holstein	48.1%	53.6%	An et al. 2010
Brazil	Nelore	41.5%	61.7%	Dominguez et al. 2011
Paraguay	Nelore	36.2%	61.4%	Dominguez et al. 2011
Brazil	Jersey	31.4%	51.8%	Sales et al. 2011
Australia	Holstein	31.6%	39.6%	Healy et al. 2013
USA	Holstein	38%	68%	Mallory et al. 2013
USA	Jersey	37.6%	-	Lucena et al. 2014
Australia	Holstein	40.3%	56.0%	Noonan et al. 2016

Table 2. **Efficiency of sexed semen use in dairy and beef cows in different geographic regions**

Country	Breeds	Type of semen		References
		Sexed	Unsexed	
USA	Angus	23%	67%	Doyle et al. 1999
Switzerland	Brown Swiss, Red Holstein	23.8%	26.6%	Bodmer et al. 2005
Finland	Holstein-Friesian	21%	46%	Andersson et al. 2006
USA	Angus	57%	76%	Seidel, Schenk 2008
USA	Holstein	40.5%	55.6%	Schenk et al. 2009
USA	Jersey	37%	-	DeJarnette et al. 2009
USA	Holstein	23%	31%	DeJarnette et al. 2010a
USA	Holstein	25%	30%	Norman et al. 2010
Brazil	Nelore	41.8%	51.8%	Sales et al. 2011
Paraguay	Nelore	39.1%	52.3%	Dominguez et al. 2011
Brazil	Nelore	45.9%	54.7%	Sà Filho et al. 2012
Brazil	Holstein x Gir	23.2%	-	Sà Filho et al. 2013
Turkey	Holstein	25.7%	39.0%	Karakaya et al. 2014
USA	Jersey	43.1%	-	Lucena et al. 2014
Brazil	Nelore, Angus	30%	-	Pellegrino et al. 2016

Despite improvements in the sorting technology, by reducing the impact of pressure, speed and stain concentrations on sperm, and replacement of laser sources and power (Schenk et al. 2009; Sharpe, Evans 2009; Arruda et al. 2012), the fertility of sexed semen is still lower than acceptable levels.

The fertilising ability of semen is a major contributor to conception. Morphology, the integrity of DNA, and the ability of sperm to maintain embryonic development dictate the reproductive potential of a bull (Saacke 2008). The fertility of sexed semen depends mostly on the susceptibility of sperm to the sorting procedure (Suh et al. 2005; Frijters et al. 2009). The exposure of sperm to ultraviolet radiation and Hoechst/laser interaction is associated with the damage of DNA (Boe-Hansen et al. 2005; Blondin et al. 2009). The reduction of sperm with damaged DNA after sorting has been found in individual bulls, suggesting a beneficial effect on the quality of semen (Blondin et al. 2009; Gosálvez et al. 2011). In studies on compensation for compromised sorted sperm by dosage increase, an improvement in conception rates with dosages of 3.5, 5.0 or 10.0 x 10^6 was not meaningful compared with a 2.1 x 10^6 dosage. A dosage increase increased conception rates in Holstein heifers for some bulls but was not comparable to either dosage of unsexed semen. Neither bulls nor sperm dosages influenced conception rates in lactating cows (DeJarnette et al. 2008, 2009, 2010b, 2011). Due to differences between bulls in the reaction of their sperm to sorting, the fertility estimates of bulls as candidates for sperm sorting based on unsexed semen to be of little value for predicting success with sexed semen (DeJarnette et al. 2010b).

Insemination within a time frame related to ovulation appeared to be more critical for sorted than for unsorted sperm. The time of insemination with sexed semen was found to be delayed to 12-24 h after the onset of observed estrus, later than that associated with using unsexed semen, suggesting closer synchrony between the function of sorted sperm and ovulation (Seidel et al. 1999; Schenk et al. 2009). Compared to insemination at natural estrus, the use of synchronisation programmes is associated with a decrease of pregnancies (Schenk et al. 2009; Abdel-Azim 2010). After the insemination of Holstein heifers with sexed semen at estrus, induced by a single $PGF_2\alpha$ treatment or at a fixed time after synchronisation, pregnancy rates were not different but rather were lower than at spontaneous estrus (Kurykin et al. 2016). Some data indicate either an increase of pregnancies or no diminishing effect by using the synchronisation of ovulation (Sá Filho et al. 2013; Lucena at al. 2014).

A reduction in conception rates with sexed semen as a service number increased was documented in dairy heifers. A greater decrease of pregnancies was observed from the third insemination (DeJarnette et al. 2009; Norman et al. 2010; DeJarnette et al. 2011). In lactating cows, the service number appeared to have little effect on the pregnancy rate of sexed semen (DeJarnette et al. 2009, 2010). The effect of the insemination number was not found using the synchronisation of ovulation in reproductively sound Holstein cows (Karakaya et al. 2014). In Holstein cows selected for their normal clinical and reproductive statuses, pregnancy rates did not differ up to the fourth insemination with sexed semen at spontaneous estrus and tended to increase at the synchronisation of ovulation. No difference in pregnancy rates was found in those cows after insemination from 50 to 100, 101 to 150 and > 150 days in milk at spontaneous estrus or synchronising ovulation (Kurykin, unpublished). The effect of days in milk on the pregnancy rate was not significant using sexed semen in reproductively sound lactating cows (Schenk et al. 2009; Karakaya et al. 2014). An increase of pregnancies was observed after 100 days in cows of unknown reproductive status (Schenk et al. 2009).

Several studies on the production of *in vivo* and *in vitro* embryos suggested that the viability of embryos derived by using sexed semen may be compromised and that this tends to increase embryonic mortality (Sartori et al. 2004; Wilson et al. 2006; Palma et al. 2008; Larson et al. 2010). Reported data on embryonic and foetal mortality using sexed semen are controversial. The loss of pregnancies occurring after insemination between 30-40 and 60-90 days and to term is either higher or does not differ from that associated with using unsexed semen (Table 3).

Table 3. **Embryonic and foetal mortality following insemination with sexed semen in heifers and lactating cows of dairy and beef breeds**

Breeds	Type of semen		References
	Sexed	Unsexed	
Angus and Holstein heifers	8.8%	6.2%	Seidel et al. 1999
Brown Swiss and Red Holstein heifers	11.1%	0.0%	Bodmer et al. 2005
Brown Swiss and Red Holstein cows	17.2%	5.5%	Bodmer et al. 2005
Holstein-Friesian cows	3.1%	5.8%	Andersson et al. 2006
Angus cows	2.0%	5.0%	Seidel, Schenk 2008
Holstein cows	9.5%	5.5%	Schenk et al. 2009
Holstein, Jersey and Danish Red heifers	4.5%	2.3%	Borchersen, Peacock 2009
Holstein heifers	10.6%	7.4%	Chebel et al. 2010
Holstein heifers	6.1%	6.5%	Healy et al. 2013
Holstein-Friesian cows	19.1%	4.8%	Karakaya et al. 2014

The damage of DNA is associated with the exposure of sperm to ultraviolet radiation and Hoechst/laser interaction (Boe-Hansen et al. 2005; Blondin et al. 2009). An increase in the loss of pregnancies due to damaged

DNA should be associated with an increase of abortions and congenital abnormalities, as DNA-damaged sperm can fertilize oocytes at the same rate as those with intact DNA (Vazques et al. 2008). The offspring born by using sexed semen do not differ from those obtained with unsexed semen (Tubman et al. 2004; Vazques et al. 2008; Chebel et al. 2010). Despite the potential damage incurred by DNA during sorting, abortion is not a biological consequence, as the damage is minimal (Healy et al. 2014). Early embryonic mortality using sexed semen can be implicated as a factor contributing to the reduction of pregnancies. An inappropriate insemination time of sorted sperm may lead to a greater extent of the aging of sperm or oocytes before or after ovulation, and the inability to maintain the development of embryos (DeJarnette et al. 2010a). Later losses of pregnancies may contribute mainly to the effect of on-farm factors for variety of reasons.

Along with the fertility potential of a herd, reproductive management is one of the factors influencing the efficiency of insemination. Farm-to-farm differences can be greater than any effect associated with sexed semen. Pregnancy rates with sexed semen on farms with excellent management may constitute 70-80% of unsexed semen and 50-60% with marginal management (Seidel 2003; Seidel, Schenk 2008). Herds may contribute the most to variation in fertility, varying more with inseminators than with bulls, which emphasises the importance of experience (Abdel-Azim 2010). Differences in proficiency may be a function of the handling of semen and the maintenance of the viability of sperm throughout the insemination process, contributing to the variation of pregnancies, which can be detected as an effect of a herd (DeJarnette et al. 2011).

Studies on improvement of efficiency of insemination with sexed semen

The limited output of sorted sperm after sorting has restricted the number of sperm available for insemination. Previously, it was suggested that a decrease of pregnancies from a small number of sperm may be avoided by deep deposition into the uterine horn that allows a greater number of viable sperm to reach the fertilization site, compared with insemination into the uterine body (Hunter, Greve 1998). Intracornual insemination has been applied in studies on sexed semen to obtain acceptable pregnancy rates. Different timed programmes have been used for insemination with sexed semen in a number of studies. The effects of various intervals for insemination, ovarian status and occurrence, and the intensity of estrus after treatment on pregnancy rates have been studied on heifers and lactating cows of different breeds.

Site of semen deposition

In a study on Angus cows, synchronised by GnRH and $PGF_2\alpha$ treatments, Doyle et al. (1999) deposited half of 1 x 10^6 frozen-thawed or half of 5 x 10^5 cooled-to-18°C sorted sperm into each uterine horn 6 to 26 h after observed estrus. The pregnancy rates for both doses were lower than after insemination into the uterine body of 1 x 10^6 or 40 x 10^6 non-sorted sperm (23 and 25% vs. 49 and 67%). Seidel et al. (1999) studied the efficiency of the bilateral deposition of 1.5 or 3.0 x 10^6 frozen-thawed sorted sperm on Holstein and Angus heifers. Heifers were synchronized by $PGF_2\alpha$, MGA/$PGF_2\alpha$ or GnRH/$PGF_2\alpha$ treatments and inseminated 12 to 24 h after observed estrus. Among 11 trials, the pregnancy rates ranged from 33 to 68%, or from 70 to 90% of the rates obtained with unsexed semen. In another study on Holstein heifers, Seidel and Schenk (2008) obtained similar pregnancy rates (43 and 44%) after the bilateral deposition of sexed semen and insemination into the uterine body at observed synchronised estrus.

In other studies on sexed semen, a unilateral deposition of 2.1 x 10^6 sorted sperm on the side with a presumed pre-ovulatory follicle was applied at spontaneous estrus and at a fixed time after the synchronisation of estrus or ovulation.

Andersson et al. (2006) reported that, despite the deposition of sexed semen into the uterine horn ipsilateral to the side of impending ovulation at spontaneous estrus in Holstein-Friesian cows, the pregnancy rate was significantly less than from the conventional deposition of unsexed semen (21 vs. 46%). In a study by Kurykin et al. (2007) on Holstein heifers, sexed semen was deposited 80-82 h after the synchronisation of estrus by $PGF_2\alpha$ into the uterine horn ipsilateral to the dominant follicle or into the uterine body. No significant difference was found in pregnancy rates (44.1 vs. 41.8%) between deposition sites.

Brogliatti et al. (2009) evaluated the effect of a timed intracornual insemination of sexed semen after the synchronization of ovulation in Holstein heifers. The protocol consisted of the intravaginal insertion of a progesterone-delivery (P4) device, $PGF_2\alpha$ and estradiol benzoate (EB) treatments. An interaction effect was found between the time and site of semen deposition. From insemination at 58 h after device removal into the uterine horn ipsilateral to the ovulatory follicle, the pregnancy rate was higher than from inseminating into the uterine body (49.2 vs. 33.6%) and into either site at 52 h (35.5 and 35.7%). An et al. (2012) compared the intracornual deposition of 2 x 10^6 sorted or non-sorted sperm 10 to 14 h after the onset of spontaneous estrus in Holstein heifers. No significant difference in pregnancy rates was found (52.8 vs. 57.7%) between both types of sperm.

In a study by Sá Filho et al. (2012), the effect of a site of sexed semen deposition at a fixed time after the synchronisation of ovulation was evaluated on Nelore cows. The protocol included an intravaginal P4 insert, EB, $PGF_2\alpha$, ECG and EB treatments followed by insemination 60-64 h after insert removal. The obtained pregnancy rates were not significantly different after insemination into the uterine body or into the uterine horn ipsilateral to the larger follicle (54% and 50%). Karakaya et al. (2014) suggested that the synchronization of ovulation in reproductively sound Holstein cows and intracornual insemination would eliminate the difference in pregnancy rates between sexed and unsexed semen. An

Ovsynch 56-h protocol was applied on cows with a corpus luteum and a follicle ≥ 10 mm in ovaries. Insemination 16-18 h after treatment into the uterine horn ipsilateral to the dominant follicle using sexed semen resulted in significantly lower pregnancy rate compared with unsexed semen (31.8 vs. 40.9% on Day 31 and 25.7 vs. 39.0% on Day 62).

Timing of insemination and expression of estrus

In a study by Kurykin et al. (2007) on Holstein heifers, estrus was synchronised by $PGF_2\alpha$ 14 days apart, and at the time of insemination, it was evaluated as strong or weak. A relationship was found between the intensity of estrus and the pregnancy rate. At 80-82 h after $PGF_2\alpha$ treatment, estrus was expressed in 88.5% heifers, which had a significantly higher pregnancy rate (45.9 vs. 20.8%) compared to that when estrus was not clearly apparent.

Sá Filho et al. (2010) aimed to improve the pregnancy rate using sexed semen by GnRH treatment at the insemination of Jersey heifers at $PGF_2\alpha$ synchronised estrus and at various intervals from the onset of estrus to insemination. Treatment with GnRH did not improve the pregnancy rate compared with non-treated heifers (47.2-53.1% vs. 48.6-51.7%). A significant increase of pregnancies occurred through the delay of insemination after the onset of estrus (37.7% at 12 to 16 h vs. 51.8% at 16.1 to 20 h vs. 55.6% at 20.1 to 24 h).

The efficiency of a timed insemination with sexed semen at various intervals after the synchronization of ovulation in Jersey heifers and Nelore cows was evaluated by Sales et al. (2011). Jersey heifers received intravaginal P4 insert, EB, $PGF_2\alpha$, and EB treatments and were inseminated at 54 or 60 h after device removal. The pregnancy rate was greater from insemination with sexed semen at 60 h than that at 54 h (31.4 vs. 16.2%). In Nelore cows, ovulation was synchronised by intravaginal P4 insert, EB, $PGF_2\alpha$, ECG and EB treatments. Cows were inseminated at 36, 48 or 60 h after device removal and examined every 12 h by ultrasonography to detect the time of ovulation. A significant increase of pregnancies was found when insemination was delayed (5.8% at 36 h vs. 20.8% at 48 h vs. 30.9% at 60 h) or when inseminating close to ovulation (37.9% > 0 to 12 h before ovulation vs. 19.4% > 12 to 24 h vs. 5.8% > 24 h).

Sá Filho et al. (2012) studied the effect of the occurrence of estrus on a timed insemination with the sexed semen of Nelore cows after the synchronisation of ovulation. Cows received intravaginal P4 insert, EB, $PGF_2\alpha$, ECG and EB treatments and were inseminated 60-64 h after insert removal. Between insert removal and insemination the estrus was expressed in 74.7% cows, which had a greater pregnancy rate than did those that did not express estrus (50 vs. 33.9%). Mallory et al. (2013) aimed to achieve an equivalent pregnancy rate with sexed and unsexed semen in Holstein heifers through the Show-Me-Synch protocol. The protocol consisted of the intravaginal insertion of a P4 device, treatment with $PGF_2\alpha$ after device removal and GnRH at insemination 66 h later. However, sexed semen resulted in fewer pregnancies than did unsexed semen (38 vs. 68%). In the heifers that expressed estrus (53-58%), the pregnancy rate was greater than in those that failed to exhibit estrus (46 vs. 26%).

Thomas et al. (2014) hypothesised that, when estrus is not expressed after synchronisation before insemination, a later insemination with sexed semen from a standard time would improve the pregnancy rate. In beef cows, estrus was synchronised using an intravaginal P4 device, GnRH treatment and, on Day 7, $PGF_2\alpha$ treatment coinciding with device removal. Prior to insemination, the estrous response by 66 h averaged 50%. After insemination, regardless of the expression of estrus at 66 h, sexed semen resulted in lower pregnancy rate than did unsexed semen (26 vs. 56%). In the cows that failed to express estrus, the pregnancy rate with sexed semen was considerably less than that in the cows that expressed estrus (3% vs. 51%). From insemination with sexed semen at 66 h at expressed estrus or 20 h after 66 h in the cows that failed to express estrus, pregnancy rates were not significantly different (42 vs. 36%). Insemination with sexed semen 20 h after 66 h in the cows that failed to express estrus resulted in a higher pregnancy rate (36%) than that at 66 h (3%), and it was comparable to that achieved using unsexed semen at 66 h (37%).

Noonan et al. (2016) compared a timed insemination with sexed or unsexed semen after the synchronisation of estrus in Holstein and Holstein-cross heifers at 47 or 51.4 h after P4 device removal. The overall conception rate for sexed semen was lower compared with unsexed semen (40.3 vs. 56%). Insemination at 52 h resulted in a higher conception rate (46 vs. 37.5%) than at 47 h. The heifers that expressed estrus prior to insemination (81%) had a considerably higher conception rate than did those that failed to express estrus (47.5 vs. 13.3%). In a study by Bombardelli et al. (2016), lactating Jersey cows were treated with $PGF_2\alpha$, followed by the monitoring of activity and the expression of estrus to detect the association of the optimal interval from the peak activity to insemination with the pregnancy rate. The extended interval from reaching activity and insemination with sexed semen was associated with a significant increase of pregnancies (≤ 3 h 20.0% vs. 4-12 h 30.6% vs. 13-22 h 44.3% vs. 23-41 h 48.9%). In addition, the occurrence of estrus upon hormonal treatments, the expression intensity and a period of high activity are associated with increased rates of synchronisation of ovulation and an improved uterine environment (Aungier et al. 2015; Pereira et al. 2016). Ovulation risk was found to be 94-96% at expressed estrus and 62-77% in the absence of estrus following treatments (Sauls et al. 2017).

Concluding remarks

Based on data from reported studies, an increase in the efficiency of sexed semen use can be achieved by combining programmes, providing the control of follicular growth, luteolysis and ovulation with timed insemination, expressed estrus and the delay of insemination close to ovulation. The reported results seem to imply that there is no positive effect of sexed semen deposition into the uterine horn. However, evidence exists that the intracornual deposition of sexed semen on the side of impending ovulation may interact with the time of insemination in leading to an increase of pregnancies. The results of recent studies suggest that, to maximise the pregnancy rate, the insemination of dairy or beef cattle with sexed semen should occur closer to presumptive ovulation. Postponed insemination after treatment from the recommended times in timed programmes by 5 to 12 h in heifers increased pregnancy rates up to 14-18%. Through delaying insemination by 12 to 24 h in lactating cows, the differences achieved were 15-33%. By applying timed programmes, up to 50-58% of treated heifers or cows may fail to express estrus before a scheduled time of insemination. The expression and intensity of estrus appeared to be associated with the pregnancy rate. After timed insemination with sexed semen at expressed estrus, the differences between pregnancy rates – 20 to 25% in heifers and 16 to 48% in lactating cows – have been shown compared to insemination when estrus was not apparent. The longer delay of insemination with sexed semen of cows that failed to express estrus before the scheduled time appeared to result in a pregnancy rate comparable to that in expressed estrus cows.

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