Eurasian Journal
of Veterinary Sciences

www.eurasianjvetsci.org

RESEARCH ARTICLE

Determination of the effect of different synchronization protocols on fetal sex in heifers

Mehmet Buğra Kıvrak[1], İbrahim Aydın[2]*, Ahmet Semacan[2], Yusuf Ziya Çakır[3]

[1]Cumhuriyet University, Faculty of Veterinary Medicine, Obstetrics and Gynecology, Sivas, Turkey
[2]Selcuk University, Faculty of Veterinary Medicine, Obstetrics and Gynecology, Konya, Turkey
[3]Agriculture and Rural Development Support Institution, Mersin, Turkey
Received:28.03.2018, Accepted: 17.09.2018
* iaydin@selcuk.edu.tr

Düvelerde farklı senkronizasyon yöntemlerinin fetal cinsiyet üzerine etkisinin belirlenmesi

Eurasian J Vet Sci, 2018, 34, 4, 284-289
DOI: 10.15312/EurasianJVetSci.2018.212

Öz

Amaç: Sunulan çalışma, düvelerde uygulanan farklı senkronizasyon yöntemlerinin fetal cinsiyet üzerine etkilerinin belirlenmesi amacıyla yapıldı.

Gereç ve Yöntem: Düveler (n=120) rastgele 3 gruba ayrılarak birinci grup (n=40) PGF2α, ikinci grup (n=40) ovsynch protokolü, 3. grup (n=40) ise intravajinal progesteron salan bir aygıt kullanılarak senkronize edildi. Gebe düveler takip edilerek buzağıların cinsiyetleri kaydedildi. Bunlara ek olarak daha iyi bir değerlendirme yapabilmek amacıyla bir önceki yıla ait işletme kayıtları alındı.

Bulgular: Birinci gruptaki düvelerde % 42,5 oranında gebelik elde edildi ve bu gebelikler sonrası şekillenen doğumlardan 11'inde erkek, 6'sında dişi buzağı doğdu. İkinci gruptaki düvelerde % 45,0 oranında gebelik elde edildi ve bu gebelikler sonrası şekillenen doğumlardan 7'sinde erkek, 11'inde dişi buzağı doğdu. Üçüncü gruptaki düvelerde ise % 40,0 oranında gebelik elde edildi ve bu gebelikler sonrası şekillenen doğumlardan 8'inde erkek, 8'inde dişi buzağı doğdu. Bir önceki yıla ait işletme kayıtları incelendiğinde gebeliği tespit edilen düvelerden % 48,1'i dişi ve % 51,9'u erkek buzağı doğduğu belirlendi. Gruplar arasında gebelik oranları ve fetal cinsiyet bakımından önemli bir farkın olmadığı tespit edildi (P>0.05). Aynı zamanda bir önceki işletme kayıtlarına göre de fetal cinsiyet bakımından istatistiki olarak bir fark belirlenemedi (P>0.05).

Öneri: Sonuç olarak, düvelerde farklı senkronizasyon yöntemlerinin fetal cinsiyet üzerine etkisinin olmadığı belirlendi. İstatistiksel fark belirlenememesine rağmen sayısal olarak çift doz PGF2α grubunda daha fazla erkek, Ovsynch grubunda ise daha fazla dişi buzağı doğduğu görüldü.

Anahtar kelimeler: Fetal cinsiyet, senkronizasyon, düve

Abstract

Aim: The aim of this study was to detect the effect of different synchronization protocols on fetal sex in heifers.

Materials and Methods: Heifers (n=120) were divided randomly into three groups. The heifers in the first group (n=40), the second group (40), and the third group (n=40) were synchronized with two doses of PGF2α, Ovsynch, and intravaginal progesterone respectively. Pregnant heifers were followed up and the sexes of calves were recorded. Operation records of the last year were taken in order for better evaluation.

Results: In the first group, the pregnancy rate was 42.7% and 11 of calves born were male, 6 of them were female. In the second group, 45% pregnancy rate was achived and 7 of calves born of these pregnancies were male, 11 of which were female. As for the third group, 40,0% pregnancy rate was achived and 8 of calves born of these pregnancies were male, 8 of them female. When the operating records belongs to previous year were analyzed, it was determined that 48,1% female calf 51,9% male calves were born. No significant differences were found in pregnancy rate and fetal sex rate between different groups (P>0.05). Moreover, there was no statistical difference in gender ratio when datas compared with operating records (P>0.05).

Conclusion: In conclusion, it was determined that different synchronization methods had no effect on fetal sex. Although there was no significant differences between groups, more male calves in PGF2α and more female calves were born in Ovsynch group.

Keywords: Fetal sex, synchronization, heifer

Introduction

It has always been intriguing to be able to manipulate the sex of offsprings and therefore a quite number of studies have been conducted on spermatozoa (Johnson and Welch 1999, Garner et al 2013) and embryos (Shea 1999, Peippo et al 2009). The sex of offsprings is of special importance in the livestock industry. Basically, male offsprings are desired in beef farms, whereas females are desired in dairy farms. Today, using sex-sorted sperm is the most commonly applied method to obtain offsprings in a desired sex. However, the high cost along with the low degrees of success of this technique limit its feasibility (Bodmer et al 2005).

Cattles produce an equal number of X and Y bearing spermatozoa and the fertilizing capability of these spermatozoa is almost the same. Expected gender ratio, thus, is 1:1 approximately (James 1996, Dominko and First 1997, Rorie 1999). However an unanticipated skewness in the gender ratio, especially in wild animal populations, has led to an idea that maternal determinants may be effective in this process. Moreover "Sex Allocation" hypothesis put forward by Trivers and Willard (1973) in their research has drawn special attention to this point, and a significant number of studies related to skewness in gender ratio due to maternal factors have been conducted on different animal species. Although mechanism underlying shift in the sex ratio has not been fully understood, it was reported in a number of studies that alteration in maternal hormone levels may change the gender ratio (James 1980, Krackow 1995, James 2008).

It is reported in the studies carried out on various animal species that in addition to genetic influence many environmental factors such as nutrition (Kent 1995), maternal psychological and physiological status (Lane and Hyde 1973), time of insemination (Martinez et al 2004), potential effect of the hormones used for synchronization (Xu and Burton 1999) can alter the gender ratio. From a broader perspective, all these factors may alter the gender ratio in two different ways; 1) affecting the period before conception 2) making one sex superior to the other. Even in the same species, contradicting results have been obtained with regards to the direct influence of the external and internal factors on gender selection (Harlap 1979, Reubinoff and Schenker 1996, Rorie et al 1999, Martinez 2004, Roelofs et al 2006, Demiral et al 2007). Therefore, it should be noted that results of these hypotheses can vary among species (Rosenfeld and Roberts 2004).

Milk yield has increased considerably in the last fifty years thanks to developing farm management systems and nutrition methods. Genetic selection and progress made for the purpose of increasing the milk yield to support dairy industry have caused a serious reduction in fertility. Synchronization methods, therefore, are widely employed throughout the world to improve reduced fertility (Walsh et al 2011, Berry et al 2016). The present study has been conducted to reveal the effect of three different synchronization methods commonly employed in the field on gender ratio.

Materials and Methods

All procedures used in this study was approved by Selcuk University Faculty of Veterinary Medicine Ethical Committee (approval number 2010/38).

The material of study was composed of 120 Holstein heifer weighing 350-400 kg under the same nutritional and environmental conditions in Bursa Karacabey Agricultural Operation. Prior to the involvement of the material into the study, health status of the heifers was evaluated through clinical and systemic examinations. Rectal palpation and ultrasonographic examination were performed to determine reproductive condition of the heifers, and all findings were recorded. Based on the results of the examinations, clinically healthy, cyclic heifers were included in the study.

The heifers were randomly divided into 3 groups. Two doses of PGF2α with 11-day interval were performed in the animals in the first group (n=40). As for the second group (n=40), Ovsynch protocol (Pursley et al 1997) was performed and the heifers in the third group (n=40) were synchronized with an internal progesterone releasing device. Without examining the animals, two doses of PGF2α (D-Cloprostenol, Dalmazin-Vetaş-Turkey), 0,15 mg each injection, with 11-day interval were administrated. Following the second injection, animals were artificially inseminated at the 60th hour. As for the second group, firstly 10 µg GnRH (Buserelin acetate, Receptal-İntervet) was administrated (day 0). On the 7th day following GnRH, 0,15 mg PGF2α was applied and two days later (day 9) 10 µg GnRH was injected. Finally, artificial insemination was performed on the day 10. An internal progesterone releasing device (1,38 g progesterone, CIDR-Pfizer) that is placed into vagina was used for the heifers in the third group for 7 days. Animals were inseminated artificially at the 56th hour after the device was taken out. Additionally, operation records of the last year which belong to animals that were detected only through estrous observation and artificially inseminated without using any synchronization method were taken in order for better evaluation of the effect of synchronization methods on fetal sex. Pregnancy, delivery rate, and gender ratio belonging to aforementioned heifers were detected with the records.

Chi-square statistical analysis was used to evaluate the obtained results based on the measurement. The research data were expressed in % means and $P < 0.05$ was considered to be important.

Results

Findings regarding the pregnancy states of heifers along with the sex of the new-born calves in the first, second, and third groups are presented in Table 1.

Table 1. Pregnancy rates and gender ratios

Groups	Pregnant heifers (%)	Female calf (%)	Male calf (%)
Group 1 (n:40)	17[a] (42,5)	6[a,A] (35,3)	11[a,A] (64,7)
Group II (n:40)	18[a] (45,0)	11[a,A] (61,1)	7[a,A] (38,9)
Group III (n:40)	16[a] (40,0)	8[a,A] (50,0)	8[a,A] (50,0)

*No significant difference was detected in same column (a) and row (A)

Table 2. Operation records belong to pervious year

Record	Female calf (%)	Male calf (%)
Pregnant heifer (n: 158)	76[a] (48,1)	82[a] (51,9)

a: There is no significant difference in same row

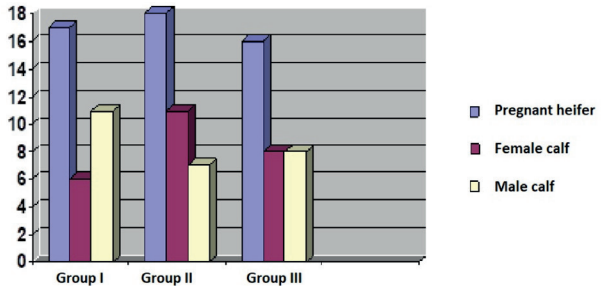

Figure 1. Number of pregnancies and sex of calves born

Pregnancy rate was 42,5% in the first group and 6 female (35,3%) and 11 (64,7%) male calves were born. A twin (female/female) was born, as well. In the second group, 45% of the heifers were diagnosed as pregnant, and they delivered. Eleven (61,1%) of 18 calves were female while 7 (38,9%) of them were male. As for the third group, pregnancy rate was found as 40%. Totally 16 calves were born from these pregnancies. Eight male (50%) and 8 (50%) female calves were born (Graph 1). When pregnancy rate after first insemination is compared between groups, no significant difference was found (P>0.05). While male and female count was equal in number in the third group, male rate in the first, female rate in the second group was higher. Although there is a difference in the count of calves, there was no significant difference in gender ratio for all groups (P>0.05). From the pregnant heifers (n: 158), who were artificially inseminated based on estrous observation without applying any synchronization methods in the light of the previous year's records, the female and male ratios were observed as 48,1% and 51,9% respectively. Just like in other groups, the results were not significantly different (P>0,05) (Table 2).

Discussion

In this study, although 3 different synchronization methods could not elicit any significant differences, there was a tendency to male in the first group using PGF2α, to female in the second group using GnRH and PGF2α. Our results were in line with those of some other studies (Whittaker ve ark. 2002, Holm 2006).

PGF2α affects a number of physiological processes such as contraction of the uterus smooth muscles, transportation of the spermatazoa, and ovulation (Duffy and Stouffer 2001, Ruan et al 2011). In some studies, which were carried out about this matter on heifers, 64% (Holm 2006) and 70% (Ostrowji et al 1988) bull calf was born. In the present study, we achieved 64% bull calf (11 males, 6 female).

As for the second group, it was observed that there was a tendency to heifer calf (61.1%). GnRH used in the Ovsynch protocol induces a sudden and high level of gonadotrophin secretion. The studies conducted on this subject yielded conflicting results. More heifer calves were born as a result of early (0. hour) and late (32. hour) inseminations performed after the last GnRH injection in a field trial carried out by Pursley et al (1997). In another study, more bull calves were born with the inseminations in the 24th hour following last GnRH injection (Youssefi et al 2013). On the other hand, there are also studies reporting that time of insemination does not affect the gender ratio (Bayril and Yılmaz 2012). Here, artificial inseminations were performed in the 24th hour following the last GnRH injection and more heifer calves were born. In the aforementioned study, lactating cows were used;

however, the present study was conducted on heifers. Estrus cycle along with the period of dominance of the dominant follicle is shorter in heifers than cows (Wolfenson et al 2004). Probably when artificial insemination in the 24th hour following GnRH administration was performed, ovulation had already occurred in heifers. Therefore, these late inseminations could be the reason why there is a tendency to female born in our study.

With regards to the issue of how progesterone synchronization affects the gender ratio, results obtained from different studies are not constant. In a study using progesterone + estradiol benzoate for synchronization, early inseminations (23-26th hour) favored male calf, whereas late (29-33th hour) inseminations favored females (Whittaker et al 2002). Additionally, although bias towards female was observed in some studies evaluating progesterone synchronization, no significant difference was detected (Xu and Burton 1999, Rivera et al 2005, Lamb et al 2014). Here, the number of male and female calves was equal in the group synchronized with progesterone. In studies of in-vitro fertilization, it is reported that when oocyte developed in an environment where androgen level is high, it especially chooses the Y bearing spermatozoa. (Grant and Irwin 2005, Grant et al 2008, Macaulay et al 2012). Furthermore, estrogen exposure causes a tendency to female in the studies conducted on different animal species (Engelhardt et al 2004, Freedberg et al 2006). The difference between our study and the aforementioned studies are probably due to the use of progesterone preparation combined with estrogen. Because the use of estrogen and other steroid hormones as growth promoters in animals intended for human consumption is prohibited in all states of the European Union, we used an intravaginal device preparation without estrogen.

There are considerable amount of studies arguing that the reason underlying alteration in the sex ratio in relation to environmental factors is that X and Y bearing spermatozoa have different morphologic characteristics (Johnson 1995, Wehner et al 1997, Pursley et al 1997, Martinez et al 2004). This piece of information that initially appeared in the two articles published by Shetter in the 1960s inspired the authors of the aforementioned studies. In the following years, the developing methods such as CASA (Computer-assisted sperm analysis) and In situ hybridization proved that X and Y bearing spermatozoa are morphologically indistinguishable (Moruzzi et al 1988, Hossain et al 2001, Grant 2006, Zavaczki et al 2006). Some new products arising as a result of the enhancing technology have continued to crystalize the issue. A study recently conducted with the aid of the atomic force microscope, which is a high-resolution device, has revealed that spermatozoa bearing X and Y chromosome coextend are similar in volume; however, they topographically differ in terms of morphological characteristics (Carvalho et al 2013). As a support for the study above, it was determined that X and Y bearing spermatozoa have different structural proteins (Chen et al 2012, De Canio et al 2014). Above all, a recent work suggested that X and Y bearing spermatozoa have different functional properties. The experiment designed by Almiñana et al (2014) obviously showed that sex specific signals produced by spermatozoa in the oviduct can be distinguished by the female. As a result of these studies, it was proven that the oviduct, in which processes such as fertilization, capacitation and early embryonic development are achieved, is an active participant of the gender selection process, not passive. The fact that, being a determinant of gender selection, the oviduct, which morphologically, biochemically, and physiologically changes in a marked way by the stimulation of steroid hormones throughout the estrous cycle, clearly shows that the hormonal status of the mother during estrus cycle may also have an effect on the sex of the offsprings (Pérez-Cerezales et al 2017, Binelli et al 2018).

Conclusion

In conclusion, though, in the present study no significant differences have been observed in the effect of hormonal changes in mothers on the sex ratio, one may reach a conclusion based on the recent studies that there is still a need for more comprehensive clinical and molecular studies.

References

Almiñana C, Caballero I, Heath PR, Maleki-Dizaji S, Parrilla I, Cuello C, Gil MA, Vazquez JL, Vazquez JM, Roca J, Martinez EA, Holt WV, Fazeli A, 2014. The battle of the sexes starts in the oviduct: modulation of oviductal transcriptome by X and Y-bearing spermatozoa. BMC genomics, 15(1), 293.

Bayril T, Yilmaz O, 2013. Effect of timing of artificial insemination after synchronization of ovulation on reproductive performance in Holstein dairy cows. Tropical animal health and production. 45(2), 411-416.

Berry DP, Friggens NC, Lucy M, Roche JR, 2016. Milk production and fertility in cattle. Annual review of animal biosciences. 4, 269-290.

Binelli M, Gonella-Diaza AM, Mesquita FS, Membrive CMB 2018. Sex Steroid-Mediated Control of Oviductal Function in Cattle. Biology. 7(1), 15.

Bodmer M, Janett F, Hässig M, Den Daas N, Reichert P, Thun R, 2005. Fertility in heifers and cows after low dose insemination with sex-sorted and non-sorted sperm under field conditions. Theriogenology. 64(7), 1647-1655.

Carvalho JO, Silva LP, Sartori R, Dode MA 2013. Nanoscale differences in the shape and size of x and y chromosome-bearing bovine sperm heads assessed by atomic force microscopy. PloS one, 8(3), e59387.

Chen X, Zhu H, Wu C, Han W, Hao H, Zhao X, Du W, Qin T, Liu Y, Wang D, 2012. Identification of differentially expressed proteins between bull X and Y spermatozoa. Journal of proteomics. 77, 59-67.

De Canio M, Soggiu A, Piras C, Bonizzi L, Galli A, Urbani A, Roncada P, 2014. Differential protein profile in sexed bovine semen: shotgun proteomics investigation. Molecular BioSystems. 10(6), 1264-1271.

Demiral O, Ün M, Abay M, Bekyürek T, 2007. The effect of artificial insemination timing on the sex ratio of offspring and fertility in dairy cows. Turkish Journal of Veterinary and Animal Sciences. 31(1), 21-24.

Dominko T, First NL, 1997. Relationship between the maturational state of oocytes at the time of insemination and sex ratio of subsequent early bovine embryos. Theriogenology, 47,1041-1050.

Duffy DM, Stouffer RL, 2001. The ovulatory gonadotrophin surge stimulates cyclooxygenase expression and prostaglandin production by the monkey follicle. MHR: Basic science of reproductive medicine. 7(8), 731-739.

Freedberg S, Bowden RM, Ewert MA, Sengelaub DR, Nelson CE 2006. Long-term sex reversal by oestradiol in amniotes with heteromorphic sex chromosomes. Biology letters, 2(3), 378-381.

Garner DL, Evans KM, Seidel GE, 2013. Sex-sorting sperm using flow cytometry/cell sorting. Spermatogenesis: Methods and Protocols, 927, 279-295.

Garner DL, Siedel GE, 2008. History of commercializing sexed semen for cattle. Theriogenology. 69:866-895

Grant VJ, 2006. Entrenched misinformation about X and Y sperm. Bmj, 332(7546), 916.

Grant VJ, Irwin RJ 2005. Follicular fluid steroid levels and subsequent sex of bovine embryos. Journal of Experimental Zoology Part A: Ecological and Integrative Physiology, 303(12), 1120-1125.

Grant VJ, Irwin RJ, Standley NT, Shelling AN, Chamley LW, 2008. Sex of bovine embryos may be related to mothers' preovulatory follicular testosterone. Biology of reproduction. 78(5), 812-815.

Harlap S, 1979 Gender of infants conceived on different days of the menstrual cycle. New Engl J Med, 300:1445–1448.

Holm DE, 2006. The economic effect of an oestrus synchronisation protocol using prostaglandin and reproductive tract scoring in beef heifers in South Africa. PhD Thesis. University of Pretori, Pretoria.

Hossain AM, Barik S, Kulkarni PM 2001. Lack of significant morphological differences between human X and Y spermatozoa and their precursor cells (spermatids) exposed to different prehybridization treatments. Journal of andrology, 22(1), 119-123.

James WH, 1980. Gonadotrophin and the human secondary sex ratio. BMJ 281 711–712.

James WH, 1996. Evidence that mammalian sex ratios at birth are partially controlled by parental hormone levels at the time of conception. Journal of Theoretical Biology, 180, 271-286

James WH, 2008. Further evidence that mammalian sex ratios at birth are partially controlled by parental hormone levels around the time of conception. Human Reproduction, 19,6,1250-1256.

Johnson LA, 1995. Sex preselection by flow cytometric separation of X and Y chromosome-bearing sperm based on DNA difference: a review. Reproduction, Fertility and Development, 7(4), 893-903.

Johnson LA, Welch GR 1999. Sex preselection: high-speed flow cytometric sorting of X and Y sperm for maximum efficiency. Theriogenology, 52(8), 1323-1341.

Kent JP, 1995. Birth sex ratios in sheep over nine lambing seasons: years 7–9 and the effects of ageing. Behavioral ecology and sociobiology, 36(2), 101-104.

Krackow S, 1995. Potential mechanisms for sex ratio adjustment in mammals and birds. Biological Reviews, 70(2), 225-241.

Lamb GC, Larson JE, Geary TW, Stevenson JS, Johnson SK, Day MLRP, Ansotegui DJ, Kesler JM, DeJarnette, Landblom DG, 2014. Synchronization of estrus and artificial insemination in replacement beef heifers using gonadotropin-releasing hormone, prostaglandin F, and progesterone. Journal of animal science, 84(11), 3000-3009.

Lane EA, Hyde TS 1973. Effect of maternal stress on fertility and sex ratio: a pilot study with rats. Journal of abnormal psychology, 82(1), 78.

Martinez F, Kaabi M, Martinez-Pastor F, Alvarez M, Anel E, Boixo JC, Paz P, De Anel L, 2004. Effect of the interval between estrus onset and artificial insemination on sex ratio and fertility in cattle: a field study. Theriogenology, 62(7), 1264-1270.

Moruzzi JF, Wyrobek AJ, Mayall BH, Gledhill BL, 1988. Quantification and classification of human sperm morphology by computer-assisted image analysis. Fertility and sterility, 50(1), 142-152.

Ostrowsji JEB, 1988. Pasture breeding of oestrus-synchronized beef heifers and its apparent effect on sex ratio of offspring. Dtsch Tierarztl Wochenschr, 95, 365-368.

Peippo J, Vartia K, Kananen-Anttila K, Räty M, Korhonen K, Hurme T, Myllymäki H, Mäki-Tanila A, 2009. Embryo production from superovulated Holstein-Friesian dairy heifers and cows after insemination with frozen-thawed sex-sorted X spermatozoa or unsorted semen. Animal reproduction science, 111(1), 80-92, 262-276.

Pérez-Cerezales S, Ramos-Ibeas P, Acuña OS, Avilés M, Coy P, Rizos D, Gutiérrez-Adán A, 2017. The oviduct: from sperm selection to the epigenetic landscape of the embryo. Biology of reproduction 98 (3), 262-276 .

Pursley JR, Wiltbank MC, Stevenson JS, Ottobre JS, Garverick HA, Anderson LL, 1997. Pregnancy rates per artificial insemination for cows and heifers inseminated at a synchronized ovulation or synchronized estrus1. Journal of dairy science, 80(2), 295-300.

Pursley JR, Silcox RW, Wiltbank MC 1998. Effect of time of artificial insemination on pregnancy rates, calving rates, pregnancy loss, and gender ratio after synchronization of ovulation in lactating dairy cows. Journal of dairy science, 81(8), 2139-2144.

Reubinoff B, Schenker J, 1996. New advances in sex preselection. Fertil Steril; 66:343–350.

Rivera, H, Lopez H, Fricke PM, 2005. Use of intravaginal progesterone-releasing inserts in a synchronization protocol before timed AI and for synchronizing return to estrus in Holstein heifers. Journal of dairy science, 88(3), 957-968.

Roelofs JB, Bouwman EB, Pedersen HG, Rasmussen ZR, Soede NM, Thomsen PD, Kemp B, 2006. Effect of time of artificial insemination on embryo sex ratio in dairy cattle. Animal reproduction science, 93(3), 366-371.

Rorie RW, 1999. Effect of timing of artificial insemination on sex ratio. Theriogenology, 52:1273–80.

Rorie RW, Lester TD, Lindsey BR, Mc New RW, 1999. Effect of timing of artificial insemination on gender ratio in beef cattle. Theriogenology, 52, 1035-1041.

Rosenfeld CS, Roberts RM 2004. Maternal diet and other factors affecting offspring sex ratio: a review. Biology of reproduction, 71(4), 1063-1070.

Ruan YC, Zhou W, Chan HC, 2011. Regulation of smooth muscle contraction by the epithelium: role of prostaglandins. Physiology, 26(3), 156-170.

Shea BF, 1999. Determining the sex of bovine embryos using polymerase chain reaction results: a six-year retrospective study. Theriogenology, 51(4), 841-854.

Trivers RL, Willard DE, 1973. Natural selection of parental ability to vary the sex ratio of offspring. Science, 179(4068), 90-92.

Von Engelhardt N, Dijkstra C, Daan S, Groothuis TG, 2004. Effects of 17-β-estradiol treatment of female zebra finches on offspring sex ratio and survival. Hormones and Behavior, 45(5), 306-313.

Walsh SW, Williams EJ, Evans ACO, 2011. A review of the causes of poor fertility in high milk producing dairy cows. Animal reproduction science, 123(3), 127-138.

Wehner GR, Wood C, Tague A, Barker D, Hubert H, 1997. Efficiency of the OVATEC unit for estrus detection and calf sex control in beef cows. Anim. Reprod. Sci. 46, 27–34.

Whittaker PR, Colazo MG, Martínez MF, Kastelic JP, Mapletoft RJ, 2002. New or used CIDR-B devices and estradiol benzoate, with or without progesterone, for fixedtime AI in beef cattle. Theriogenology, 57, 391.

Wolfenson D, Inbar G, Roth Z, Kaim M, Bloch A, Braw-Tal R, 2004. Follicular dynamics and concentrations of steroids and gonadotropins in lactating cows and nulliparous heifers. Theriogenology, 62(6), 1042-1055.

Xu ZZ, Burton LJ, 1999. Reproductive performance of dairy heifers after estrus synchronization and fixed-time artificial insemination. Journal of dairy science, 82(5), 910-917.

Youssefi R, Vojgani M, Gharagozlou F, Akbarinejad V, 2013. More male calves born after Presynch-Ovsynch protocol with 24-hour timed AI in dairy cows. Theriogenology, 79(5), 890-894.

Zavaczki Z, Celik-Ozenci C, Ovari L, Jakab A, Sati GL, Ward DC, Huszar G, 2006. Dimensional assessment of X-bearing and Y-bearing haploid and disomic human sperm with the use of fluorescence in situ hybridization and objective morphometry. Fertility and sterility, 85(1), 121-127.