



Effect of embryo quality and developmental stages on pregnancy rate during fresh embryo transfer in beef heifers

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Abstract

The objective of this study was to evaluate the effect of embryo quality and developmental stages on pregnancy rate in beef heifer recipients. The present study used 168 Simmental breed cows as donors, and 618 beef cattle breed heifers as recipients. The quality and developmental stages of the collected embryos were evaluated according to the criteria specified by the International Embryo Technology Society. Accordingly, the embryos in the compact morula, early blastocyst, blastocyst, and expanded blastocyst stages that were of Code I (excellent) and Code II (good) quality levels were transferred as fresh embryos to the recipient heifers. Prior to the transfer, the recipients were synchronized using the Ovsynch protocol, and the embryos obtained were transferred to 618 beef heifers. Pregnancy examinations were performed on days 30 and 60. On day 30, the pregnancy rates with Code I and Code II embryos were determined as 44.15% and 32.58%, respectively. According to the developmental stages, the pregnancy rates with Code I quality compact morula, early blastocyst, blastocyst, and expanded blastocyst were determined as 44.64%, 45.67%, 45.83%, and 33.33%, respectively. The rates of pregnancy with Code II quality compact morula, early blastocyst, and blastocyst were determined as 32.03%, 32.14%, and 50.0%, respectively. In conclusion, the pregnancy rates with Code I quality embryos were found to be higher compared with Code II embryos ($P < 0.05$). It was also determined that the embryonic developmental stages had no effect on the pregnancy rate ($P > 0.05$).

Keywords Heifer · Bovine embryos · Embryo quality · Stage · Pregnancy rate

Introduction

Embryo transfer is a widely used biotechnological technique that aims to increase the number of animals with high yield and superior genetic capacity (Hasler, 2014). Embryo transfer is also used for the genetic testing of bulls, disease control, shortening of generation interval, import and export purposes, and for improving fertility in repeat breeder cows (Siedel and Siedel, 1991; Mapletoft and Bo, 2016). According to data from the International Embryo Technology Society (IETS),

approximately one million embryo transfers per year have been performed in cows for the last few years. Cattle embryos can be obtained in vivo or in vitro and are transferred to the recipient animals as either fresh or frozen and thawed embryos. Extensive studies have been conducted for increasing the efficiency of these methods and the rates of pregnancy and for identifying the factors affecting these rates (Ferraz et al., 2016; Perry, 2017; Julon et al., 2018).

Reportedly, conception rates observed following the transfer of transferrable embryos from donor animals to suitable recipients vary between 30 and 60% (Spell et al., 2001; Smith and Grimmer, 2002; Hasler, 2004; Vasconcelos et al., 2011; Mapletoft and Bo, 2016). The low or high conception rates are affected by various factors such as the developmental stage and quality of the embryo, site of embryo deposit in the uterus, difficulty level of the transfer, the use of frozen or fresh embryo, experience of the operator, the quality of the *corpus luteum*, the use of a heifer or cow, the use of a beef or dairy cow, and the season of transfer (Hasler, 2004; Bényei et al., 2006; Looney et al., 2006; Nogueira et al., 2012; Ongubo et al., 2015; Ferraz et al., 2016; Julon et al., 2018; Roper

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et al., 2018). Studies report that the highest rate of conception in recipient cattle following embryo transfer occurs with the transfer of excellent and good quality embryos (Putney et al., 1988; Bényei et al., 2006; Ferraz et al., 2016).

The objective of this study was to evaluate the effect of embryo quality and developmental stages on conception rate at in vivo embryo production in beef heifers.

Materials and methods

The study was conducted with the approval and permission of the Ethics Committee of Selcuk University Faculty of Veterinary Medicine, Experimental Animals Production and Research Center (Approval Number: 2018/99).

Location

This study was conducted between May 2017 and June 2018 at the Gozlu Agriculture Enterprise (38°29'42"N, 32°27'11"E) affiliated with the General Directorate of Agricultural Enterprises (TİGEM) located in the province of Konya, Turkey. According the management system of the enterprise, the animals are kept in separate groups in free-range paddocks, each housing 150 animals. The animals are regularly vaccinated for Brucella, IBR, BVD, enterotoxemia, foot-and-mouth disease of cattle, variola, and fungal infections. The rations of animals are prepared with total mix ration, and the animals are fed twice daily. The ration contains corn silage, clover silage, dry clover, vetch, triticale, hay, concentrate feed, and vitamin and mineral supplements.

Donors and recipient animals

In this study, 168 Simmental breed cows aged 2.5 to 4 years, body condition score of 3–3.5, and between 60 and 150 days postpartum were used as donors. The genital organs of the donor were controlled with transrectal palpation and ultrasonographic examination (6.0 MHz linear probe, Falcovet, Pie Medical, Netherlands). The cattle with a corpus luteum in the ovary and without any problems in the uterus and cervix on examination were assigned as donors. The recipient animals comprised 618 heifers, body condition score of 5–6 aged between 16 and 24 months that belong to beef cattle breeds, such as the Angus, Limousin, Hereford, Charolais, and Belgian Blue.

Synchronization and superovulation of donors

The estrous synchronization of the donor cows was performed using a progesterone-based protocol. To this end, an intravaginal device containing progesterone (1.38 g, Eazi-Breed CIDR, Zoetis, USA) was placed inside the vagina,

and a GnRH analogue (10 µg, Buserelin Acetate, Receptal, MSD, USA) was intramuscularly treated (on day 0). For achieving superovulation, 8 decreasing doses of FSH (400 µg, Stimufol, Repröbiol, Belgium) were intramuscularly injected between days 7 and 10 while estrous synchronization was continuing. Afterward, on the morning of day 9, PGF_{2α} (25 mg, Dinoprost Tromethamine, Dinolytic, Zoetis, USA) was intramuscularly treated, and progesterone was removed from the vagina on the evening of the same day. Between 48 and 60 h after the removal of CIDR (day 11), the donors were inseminated twice.

Synchronization of recipient

An Ovsynch protocol was used for ensuring the estrous synchronization of the recipient animals. On the day the synchronization was initiated, Kamar® was attached to the sacral area of all recipients. The recipients received an intramuscular injection of GnRH on the first day (day 0), PGF_{2α} on day 6, and GnRH on day 9. After the second GnRH treatment, the heifers were monitored. Estrus was detected and recorded by observing the change in the color of Kamar®.

Uterus flushing

This procedure was performed on the seventh day after artificial insemination. Uterus flushing was performed for donors that had a total of ≥ 3 corpus luteum in both ovaries. Uterus flushing was done by applying 80 to 100 mL of lactated Ringer's solution (%1 calf serum + 200 mg kanamycin) several times (3 to 4 times). During the flushing procedure, the embryos were collected into a filter (EmCon Filter, 75 µm).

Evaluation and classification of bovine embryos

The flushed uterus material was taken onto Petri dishes and scanned under a stereomicroscope. The embryos were evaluated according to the IETS criteria (Bó and Mapletoft, 2013). Unfertilized ovum was considered stage 1, while an embryo with 2–12 cells was considered to be in stage 2, early morula as stage 3, compact morula as stage 4, early blastocyst as stage 5, blastocyst as stage 6, expanded blastocyst as stage 7, hatched blastocyst as stage 8, and expanded hatched blastocyst as stage 9.

The assessment of embryo quality was made according to morphological integrity. Code I (excellent or good) corresponded to very low levels of irregularity between the cells, a ratio of $> 85\%$ viable embryonic cells and a round and unfolded zona pellucida. Code II (fair) is characterized by a medium level of irregularity between the cells and a viable cell ratio of 50%. Code III (poor) is characterized by irregularities in the form of the embryo and a viable cell ratio of 25%. Code IV (dead or degenerated) are embryos with oocytes or dead

cells with uncompleted division. According to these criteria, Code I and Code II embryos were considered to be of transferrable quality.

Embryo transfer to recipients

The collected Code I and II embryos were taken to the holding media (ViGRO™ Holding Plus, Vetoquinol, France). Later, the embryos were transferred freshly to the recipients. On day seven of the estrous cycle, the recipients were examined by transrectal palpation and ultrasonography. In this examination, animals with ≥ 15 mm corpus luteum were identified as potential recipients. The embryo was transferred to the cranial one-third section of the cornu uteri that was located on the side where the corpus luteum was found.

Pregnancy diagnosis

The recipients underwent pregnancy examination using real-time ultrasonography device (6.0 MHz linear probe, Falcovet, Pie Medical, Netherlands) on day 30 after embryo transfer. During this examination, the observation of a hypoechogenic embryo inside a non-echogenic area of the uterus was recorded as positive for pregnancy. Heifers that were identified as pregnant were examined by ultrasonography for a second time on day 60. On the basis of these examinations, the rate of pregnancy losses between days 30 and 60 was determined. The loss of pregnancy was determined if the animals diagnosed as pregnant in the first ultrasonography lacked a fetus on the next examination (day 60) or no fetal heartbeat was observed or if the fetal fluids were reabsorbed.

Statistical analysis

Data were analyzed using the SPSS 25 (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.) software package. The variables were expressed as mean \pm standard deviation, median (maximum–minimum), percentage, and frequency. The fit of the data to the repeated-measures analysis of variance was evaluated using Mauchly's Sphericity Test and the Homogeneity Testing for Box-M Variances. Comparison of means was performed using the factorial repeated-measures analysis of variance. If the assumptions of the parametric tests (factorial repeated-measures analysis of variance) were not met, either the Greenhouse–Geisser test (Greenhouse and Geisser, 1959) with a corrected degree of freedom or the Huynh–Feldt test (Huynh and Feldt, 1976) was used. Multiple comparisons were performed using Bonferroni Correction test.

Results

One hundred forty-six out of 168 (86.9%) Simmental breed cows undergoing the study procedures responded to superovulation. A total of 618 transferrable embryos with Code I and II quality according to the IETS criteria were collected from 146 cows. Of these embryos, 351 were of Code I and 267 were of Code II.

In terms of developmental stages, 168 of Code I embryos were compact morula, 81 were early blastocysts, 72 were blastocysts, and 30 were expanded blastocysts. Of Code II embryos, 231 were compact morula, 28 were early blastocysts, and 8 were blastocysts. In both Code I and Code II embryos, the compact morula was the most frequently encountered developmental stage (64.56%) (Table 1).

The pregnancy rates at days 30 and 60 following the transfer according to the quality level of the embryos are shown in Table 2. On days 30 and 60 of pregnancy, the pregnancy rates in Code I embryos was higher compared with those in Code II embryos ($P < 0.05$). Pregnancy rates on the 30th day following the transfer of Code I and II quality embryos were 44.15 and 32.58%, respectively ($P < 0.05$). Mean pregnancy rate (when embryo quality was not evaluated) was 39.15%. No statistically significant difference was noted between the rates of pregnancy according to the developmental stages of Code I embryos (Table 3, $P > 0.05$). The pregnancy rates of Code I quality embryos on the 30th day following the transfer were found to be 44.64, 45.67, 45.83, and 33.33% for compact morula, early blastocyst, blastocyst, and expanded blastocyst, respectively ($P > 0.05$). Similarly, no statistically significant difference was noted between the pregnancy rates according to the developmental stages of Code II embryos (Table 4, $P > 0.05$). The pregnancy rates of Code II quality embryos on the 30th day following the transfer were determined as 32.03, 32.14, and 50% for compact morula, early blastocyst, and blastocyst, respectively ($P > 0.05$). When they were not classified according to quality, it was observed that the highest pregnancy rate was observed with the early blastocyst and blastocyst stages (Table 5, $P < 0.05$). Moreover, pregnancy rates of Code I quality compact morula, early blastocyst, and expanded blastocyst were found to be higher than Code II

Table 1 Classification of embryos according to quality and developmental stages

Developmental stages	Code I	Code II	Total
Compact morula	168 (27.18%)	231 (37.37%)	399 (64.56%)
Early blastocyst	81 (13.10%)	28 (4.53%)	109 (17.63%)
Blastocyst	72 (11.65%)	8 (1.29%)	80 (12.94%)
Exp. blastocyst	30 (4.85%)	-	30 (4.85%)
Total	351 (56.79%)	267 (43.20%)	618 (100.0%)

Table 2 Conception rates at 30th and 60th days following transfer according to embryo quality

Embryo quality	Total (n)	Day 30	Day 60	Pregnancy loss between 30 and 60 days
Code I	351	155 (44.15%) ^{aA}	143 (40.74%) ^{aA}	12/155 (7.74%) ^a
Code II	267	87 (32.58%) ^{bA}	74 (27.71%) ^{bA}	13/87 (14.94%) ^b
Total	618	242 (39.15%)	217 (35.11%)	25/242 (10.33%)

^{a, b} Within column and ^{A-B} within rows, percentage values with different superscript are significantly different ($P < 0.05$)

quality embryos on day 30 following the transfer ($P < 0.05$). Loss of pregnancy between days 30 and 60 following the transfer of embryos was higher among Code II quality embryos compared with Code I quality embryos ($P < 0.05$). The rate of pregnancy loss between days 30 and 60 following the transfer of Code I and II quality embryos was determined as 7.74 and 14.94%, respectively ($P < 0.05$). The rate of total pregnancy loss between days 30 and 60 following the transfer was 10.33%. No statistically significant difference was identified between the developmental stages in terms of pregnancy loss ($P > 0.05$).

Discussion

In this study, the majority of the embryos with transferrable quality (Codes I and II) collected from the donors comprised embryos in the compact morula stage. In the cattle, uterus flushing is performed on days 6–8 following estrous. The developmental stages of the collected embryos may vary depending on the day the uterus flushing is performed (Mapletoft and Bo, 2016). Uterus flushing performed on day seven after estrous resulted most frequently in compact morulas, early blastocysts, and blastocysts (Hasler, 2014; Jahnke et al., 2014). In a study conducted by Leroy et al. (2005), it was determined that most of the embryos collected after uterus flushing were in the compact morula stage and to a lesser extent in the early blastocyst and blastocyst stages. A delayed ovulation and/or a retarded development together with a delayed blastulation can be responsible for this observation. Also, whether this is associated with decreased embryo quality and viability is uncertain (Leroy et al., 2005).

Embryo quality grade is determined by visual assessment of an embryo's morphological characteristics. Characteristics used to determine the quality grade of an individual embryo include uniformity of blastomeres (size, color, and shape), presence of extruded cells, and presence of dead/degenerating blastomeres (Bó and Mapletoft, 2013; Jahnke et al., 2014). Morphological embryo grading by microscopy is a subjective practice and does not necessarily mean that all embryos graded as equal are going to be equally functionally competent (Smith and Grimmer, 2002).

Code I and Code II embryos are considered transferrable. There can be variations in the pregnancy rates following the transfer of these embryos (Bó and Mapletoft, 2013). The study of Hasler et al. (1987) determined that higher quality embryos are associated with higher pregnancy rates. In the present study, pregnancy examinations performed on day 30 revealed that Code I embryos had a higher pregnancy rate than Code II embryos (44.15 vs. 32.58%, respectively, $P < 0.05$). In a study conducted by Ferraz et al. (2016), the rates of pregnancy for heifers and non-heifer cows following the transfer of Code I embryos was found to be 45.5% and 37.3%, while the rates of pregnancy for heifers and non-heifer cows following the transfer of Code II embryos was found to be 34.6% and 30.1% ($P < 0.05$). It was thus reported that the pregnancy rate following transfer was higher with Code I embryos compared with Code II embryos. A study conducted by Bényei et al. (2006) reported pregnancy rates following the transfer of Code I and Code II embryos that were similar to those reported in the above-mentioned studies (41.9% vs 33.3%, respectively). It was concluded that lower embryo quality resulted in lower pregnancy rates. The reason for this is thought to be the lower rate of viable cells with decreasing embryo quality.

Table 3 Conception rates of Code I quality embryos at 30th and 60th days following transfer according to their developmental stages

Developmental stages	Total (n)	Day 30	Day 60	Pregnancy loss between 30 and 60 days
Compact morula	168	75 (44.64%) ^{aA}	67 (39.88%) ^{aA}	8/75 (10.66%) ^a
Early blastocyst	81	37 (45.67%) ^{aA}	35 (43.2%) ^{aA}	2/37 (5.4%) ^a
Blastocyst	72	33 (45.83%) ^{aA}	31 (43.05%) ^{aA}	2/33 (6.06%) ^a
Exp. Blastocyst	30	10 (33.33%) ^{aA}	10 (33.33%) ^{aA}	-

^{a, b} Within column and ^{A-B} within rows, percentage values with different superscript are significantly different ($P < 0.05$)

Table 4 Conception rates of Code II quality embryos at 30th and 60th days following transfer according to their developmental stages

Developmental stages	Total	Day 30	Day 60	Pregnancy loss between 30 and 60 days
Compact Morula	231	74 (32.03%) ^{aA}	64 (27.70%) ^{aA}	10/74 (13.51%) ^a
Early Blastocyst	28	9 (32.14%) ^{aA}	7 (25.0%) ^{aA}	2/9 (22.22%) ^a
Blastocyst	8	4 (50.0%) ^{aA}	3 (37.5%) ^{aA}	1/4 (25.0%) ^a

^{a, b} Within column and ^{A-B} within rows, percentage values with different superscript are significantly different ($P < 0.05$)

The present study identified no statistically significant difference between the rates of pregnancy according to the developmental stages of Code I and Code II embryos ($P > 0.05$). However, when a classification based on quality was not performed, it was found that the early blastocyst and blastocyst stages were associated with higher pregnancy rates than the compact morula and expanded blastocyst stages ($P < 0.05$). Numerous studies have described that embryonic developmental stages have no impact on pregnancy rates (Coleman et al., 1987; Hasler, 2001; Spell et al., 2001; Bényei et al., 2006; Ferraz et al., 2016;). According to Putney et al. (1988), the lowest pregnancy rate was achieved following the transfer of embryos in the morula stage, and pregnancy rate increased with advancing developmental stage of the embryos. Hasler et al. (1987) determined that the highest pregnancy rate was achieved following the transfer of embryos in the early blastocyst and blastocyst stages, and that both of these developmental stages were associated with a higher pregnancy rate than the compact morula and expanded blastocyst. Similarly, in the present study, when embryos were not classified according to their quality levels, it was found that higher pregnancy rates were achieved from embryos in the early blastocyst and blastocyst stages. The reason for higher pregnancy rate observed following the transfer of embryos in the early blastocyst and blastocyst stages is considered to be associated with their faster or earlier development (earlier ovulation) relative to the compact morula stage. On the contrary, higher pregnancy rate achieved with the early blastocyst and blastocyst stages compared with expanded blastocysts is thought to stem from the damage to the

embryos during uterus flushing or transfer (Hasler et al., 1987).

It is known from other studies that embryonic death in cattle can occur at different time points following insemination. The majority of embryonic deaths take place until day 18 (Sreenan and Diskin, 1986; Farin et al., 2004). The rate of fetal deaths after day 42 varies between 5 and 8% (Ayalon, 1978). Forar et al. (1995), on the other hand, reported the rate of fetal deaths to be ranging between 0.4% and 10.4%. Silva et al. (2002) reported that deaths between days 45 and 60 following embryo transfer can be associated with the characteristics of the recipient and the method of transfer. The present study determined a rate of pregnancy loss of 10.33% between days 30 and 60. These results are similar to the pregnancy loss rates found in the study of Silva et al. (2002) on heifers (late embryonic mortality 10.7%, early fetal mortality 2.9%, total 13.6%). Taverne et al. (2002) reported a pregnancy loss rate of 13.1% between days 32 and 52. In contrast, Sartori et al. (2006) reports higher rates of pregnancy loss between days 32 and 66 after embryo transfer.

The embryonic mortality rate in this study between days 30 and 60 of gestation (10.33%) was found quite high. Because embryo transfer programs performed on dairy breed heifers report higher pregnancy rates and lower embryonic mortality rates (Hasler, 2001; Aoki et al., 2003; Baruselli et al., 2011). The use of beef heifers as recipient animals in the present study resulted in lower pregnancy rates and higher embryonic mortality rates than expected. This is because beef cattle breeds, and especially beef heifers, are aggressive and excitable animals. The pregnancy examination performed on day 30th following the transfer and re-contact with humans could have caused the release of stress factors (cortisol, PGFM). In a

Table 5 Conception rates of Code I and Code II quality embryos on 30th day following transfer according to developmental stages

Developmental stages	Code I	Code II	Total
Compact Morula	75/168 (44.64%) ^{aA}	74/231 (32.03%) ^{aB}	149/399 (37.34%) ^a
Early Blastocyst	37/81 (45.67%) ^{aA}	9/28 (32.14%) ^{aB}	46/109 (42.2%) ^b
Blastocyst	33/72 (45.83%) ^{aA}	4/8 (50.0%) ^{aA}	37/80 (46.25%) ^b
Exp. Blastocyst	10/30 (33.33%) ^a	-	10/30 (33.33%) ^a
Total	155/351 (44.15%) ^A	87/267 (32.58%) ^B	242/618 (39.15%)

^{a, b} Within column and ^{A-B} within rows, percentage values with different superscript are significantly different ($P < 0.05$)

study involving excitable beef cattle breeds, Kasimanickam et al. 2018 reported higher levels of cortisol and PGFM and lower pregnancy rates after embryo transfer. In the present study, it is believed that the pregnancy examinations performed on the animals may have led to the release of cortisol and PGFM that cause excitement, and that this may have resulted in pregnancy losses.

Reportedly, embryo quality and developmental stage have an effect on the rate of embryonic deaths (Taverne et al., 2002; Sartori et al., 2006; Ongubo et al., 2015). A similar situation was observed in the present study. The rate of embryonic death in Code I embryos was lower compared with that in Code II embryos ($P < 0.05$). Farin and Farin (1995) reported that in vivo produced Code I embryos had a higher chance of survival than in vivo produced Code II embryos. It is known that Code I embryos are more resistant to mortality. In addition to this, well-developing embryos produce more interferon-tau during the maternal acceptance phase of the pregnancy, thereby continuing their development by preventing the release of PGF2 α (Mann and Lamming, 1999; Mann et al., 2006; Wade et al., 2008).

Conclusions

In conclusion, the present study determined that embryo quality is a determining factor for the pregnancy rate, and that while the developmental stage of the embryo does not affect the pregnancy rate. Furthermore, it was also concluded that higher quality embryos are associated with less embryonic death owing to the better development they exhibit.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- Aoki, S., Murano, S., Miyamura, M., Hamano, S., Terawaki, Y., Dochi, O., Koyama, H., 2003. Factors affecting on embryo transfer pregnancy rates of in vitro-produced bovine embryos. *Reproduction, Fertility and Development*, 16(2):206.
- Ayalon, N., 1978. A review of embryonic mortality in cattle. *Journal of Reproduction and Fertility*, 54:483–93.
- Baruselli, P.S., Ferreira, R.M., Sales, J.N.S., Gimenes, L.U., Sá Filho, M.F., Martins, C.M., Rodrigues, C.A., Bó, G.A., 2011. Timed embryo transfer programs for management of donor and recipient cattle. *Theriogenology*, 76(9):1583–93.
- Bényei, B., Komlósi, I., Pécsi, A., Pollott, G., Marcos, C.H., de Oliveira Campos, A., Lemes, M.P., 2006. The effect of internal and external factors on bovine embryo transfer results in a tropical environment. *Animal Reproduction Science*, 93(3–4):268–79.
- Bó, G.A., Mapletoft, R.J., 2013. Evaluation and classification of bovine embryos. *Animal Reproduction*, 10(3):344–8.
- Coleman, D.A., Dailey, R.A., Leffel, R.E., Baker, R.D., 1987. Estrous synchronization and establishment of pregnancy in bovine embryo transfer recipients. *Journal Dairy Science*, 70(4):858–66.
- Farin, P.W., Farin, C.E., 1995. Transfer of bovine embryos produced in vivo or in vitro: survival and fetal development. *Biology of Reproduction*, 52:676–82.
- Farin, P.W., Miles, J.R., Farin, C.E., 2004. Pregnancy loss associated with embryo technologies in cattle. In: *Proceedings of the 23rd World Buiatrics Congress, Canada*.
- Ferraz, P.A., Burnley, C., Karanja, J., Viera-Neto, A., Santos, J.E.P., Chebel, R.C., Galvão, K.N., 2016. Factors affecting the success of a large embryo transfer program in Holstein cattle in a commercial herd in the southeast region of the United States. *Theriogenology*, 86(7):1834–41.
- Forar, A.L., Gay, J.M., Hancock, D.D., 1995. The frequency of endemic fetal loss in dairy cattle: A review. *Theriogenology*, (6):989–1000.
- Greenhouse, S.W., Geisser, S., 1959. On methods in the analysis of profile data. *Psychometrika*, 24:95–112.
- Hasler, J.F., 2001. Factors affecting frozen and fresh embryo transfer pregnancy rates in cattle. *Theriogenology*, 56(9):1401–15.
- Hasler, J.F., 2004. Factors influencing the success of embryo transfer in cattle. In: *Proceedings of the 23rd World Buiatrics Congress, Canada*.
- Hasler, J.F., 2014. Forty years of embryo transfer in cattle: A review focusing on the journal *Theriogenology*, the growth of the industry in North America, and personal reminiscences. *Theriogenology*, 81(1): 152–69.
- Hasler, J.F., McCauley, A.D., Lathrop, W.F., Foote, R.H., 1987. Effect of donor-embryo-recipient interactions on pregnancy rate in a large-scale bovine embryo transfer program. *Theriogenology*, 27(1): 139–68.
- Huynh, H., Feldt, L.S., 1976. Estimation of the box correction for degrees of freedom from sample data in randomized block and split-plot designs. *Journal of Educational and Behavioral Statistics*, 1(1):69–82.
- Jahnke, M.M., West, J.K., Youngs, C.R., 2014. Evaluation of in vivo-derived bovine embryos. *Bovine Reproduction*, 733–48.
- Julon, D., Burga, N., Bardales, W.P.V., 2018. Factors affecting the pregnancy rate in transfers of embryos in crossbreed Brown Swiss. *MOJ Anatomy & Physiology*, 5(2):101–4.
- Kasimanickam, R.K., Hall, J.B., Estill, C.T., Kastelic, J.P., Joseph, C., Abdel Aziz, R.L., Nak, D., 2018. Flunixin meglumine improves pregnancy rate in embryo recipient beef cows with an excitable temperament. *Theriogenology*, 107:70–7.
- Leroy, J.L.M.R., Opsomer, G., De Vlieghe, S., Vanholder, T., Goossens, L., Geldhof, A., Bols, P.E., de Kruif, A., Van Soom, A., 2005. Comparison of embryo quality in high-yielding dairy cows, in dairy heifers and in beef cows. *Theriogenology*, 64(9):2022–36.
- Looney, C.R., Nelson, J.S., Schneider, H.J., Forrest, D.W., 2006. Improving fertility in beef cow recipients. *Theriogenology*, 65(1): 201–9.
- Mann, G.E., Lamming, G.E., 1999. The influence of progesterone during early pregnancy in cattle. *Reproduction Domestic in Animals*, 34(3–4):269–74.
- Mann, G.E., Fray, M.D., Lamming, G.E., 2006. Effects of time of progesterone supplementation on embryo development and interferon- τ production in the cow. *Veterinary Journal*, 171(3):500–3.
- Mapletoft, R.J., Bo, G., 2016. Bovine embryo transfer. In: *IVIS Reviews in Veterinary Medicine*.

- Nogueira, É., Cardoso, G.S., Junior, H.R.M., Dias, A.M., Ítavo, L.C.V., Borges, J.C., 2012. Effect of breed and corpus luteum on pregnancy rate of bovine embryo recipients. *Revista Brasileira de Zootecnia*, 41(9):2129–33.
- Ongubo, M.N., Rachuonyo, H.A., Lusweti, F.N., Kios, D.K., Kitilit, J.K., Musee, K., Tonui, W.K., Lokwaleput, I.K., Oliech, G.O., 2015. Factors affecting conception rates in cattle following embryo transfer. *Uganda Journal of Agricultural Sciences*, 16(1):19–27.
- Perry, G., 2017. 2016 statistics of embryo collection and transfer in domestic farm animals Collated. *Embryo Technology Newsletter* 35(4):8–23.
- Putney, D.J., Thatcher, W.W., Drost, M., Wright, J.M., DeLorenzo, M.A., 1988. Influence of environmental temperature on reproductive performance of bovine embryo donors and recipients in the southwest region of the united states. *Theriogenology*, 30(5):905–22.
- Roper, D.A., Schrick, F.N., Edwards, J.L., Hopkins, F.M., Prado, T.M., Young, C.D., Wilkerson, J.B., Saxton, A.M., Young, C.D., Smith, W.B., 2018. Factors in cattle affecting embryo transfer pregnancies in recipient animals. *Animal Reproduction Science*, 199:79–83.
- Sartori, R., Gümen, A., Guenther, J.N., Souza, A.H., Caraviello, D.Z., Wiltbank, M.C., 2006. Comparison of artificial insemination versus embryo transfer in lactating dairy cows. *Theriogenology*, 65(7):1311–21.
- Siedel, G.E., Siedel, S.M., 1991. Training manual for embryo transfer in cattle. *FAO Animal Production and Health*.
- Silva, J.C., Costa, L.L., Silva, J.R., 2002. Plasma progesterone profiles and factors affecting embryo-fetal mortality following embryo transfer in dairy cattle. *Theriogenology*, 58(1):51–9.
- Smith, A.K., Grimmer, S.P., 2002. Pregnancy rates for Grade 2 embryos following administration of synthetic GnRH at the time of transfer in embryo-recipient cattle. *Theriogenology*, 57(8):2083–91.
- Spell, A.R., Beal, W.E., Corah, L.R., Lamb, G.C., 2001. Evaluating recipient and embryo factors that affect pregnancy rates of embryo transfer in beef cattle. *Theriogenology*, 56(2):287–97.
- Sreenan, J.M., Diskin, M., 1986. Embryonic mortality in farm animals. Dordrecht: Martinus Nijhoff Publishers.
- Taverne, M.A.M., Breukelman, S.P., Perenyi, Z., Dieleman, S.J., Vos, P.L.A.M., Jonker, H.H., Ruigh, L., Wagtendonk-de Leeuw, J.M., 2002. The monitoring of bovine pregnancies derived from transfer of in vitro produced embryos. *Reproduction Nutrition Development*, 42:613–24.
- Vasconcelos, J.L.M., Jardina, D.T.G., Sá Filho, O.G., Aragon, F.L., Veras, M.B., 2011. Comparison of progesterone-based protocols with gonadotropin-releasing hormone or estradiol benzoate for timed artificial insemination or embryo transfer in lactating dairy cows. *Theriogenology*, 75(6):1153–60.
- Wade, M., Roche, J.F., Crowe, M.A., Fair, T., Evans, A.C.O., Duffy, P., Kenny DA, Roche JF, Lonergan P., 2008. Effect of increasing progesterone concentration from Day 3 of pregnancy on subsequent embryo survival and development in beef heifers. *Reproduction Fertility and Development*, 20(3):368.

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