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Theriogenology

Factors Affecting Pregnancy Rate Per Embryo Transfer of In Vivo Produced Embryos in Recipient Holstein Heifers

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ABSTRACT

The present study aimed to determine factors responsible for variation of pregnancy establishment after transfer of in vivo produced embryos into recipient Holstein heifers. Embryos were produced from 19 donor Holstein cows using two super-stimulation protocols, estradiol-based and GnRH-based. A total of 95 fresh transfers have been carried out into previously selected recipient heifers and the grade of transferred embryo was recorded. Pregnancy was diagnosed in recipients 30 days after transfer. Serum samples were obtained from recipients on day of transfer and their ovaries were scanned using ultrasonography to record the type of CL (compact vs cavitory). In addition, recipient data as age, body weight, body height, average daily gain as well as type of estrus (spontaneous vs induced) were retrieved from available automatic records. Pregnancy rate per embryo transfer (PR/ET) was compared among different recipient categories using chi square analysis. PR/ET did not vary between different age, body weight, body height or daily gain recipient categories (P>0.05). PR/ET was similar between recipient groups receiving embryos from GnRH-superovulated donors and those from estradiol-superovulated donors (P>0.05). Presence of cavitory CL in recipient did not affect the risk of pregnancy establishment after embryo transfer. Interestingly, PR/ET was higher (P<0.05) in recipients with induced estrus (72.73%), compared to 35.72% in those with spontaneous estrus. Similarly, PR/ET was higher in high progesterone recipients (79.50%) than that achieved by low progesterone recipients (55.40%). Further, first grade embryo achieved greater (P<0.05) PR/ET than that of second and third grade embryo (79.17 vs 48.94%, respectively). Conceivably, the type of recipient CL did not affect PR/ET, while heifers with higher blood progesterone at embryo transfer are better selected as embryo recipients.

Keywords: *Embryo transfer, recipient factors, pregnancy rate, Holstein heifers.*

INTRODUCTION

Embryo transfer technology is an important breeding technique which aids in fortification of genetic superiority of cattle herds for both improved production and reproduction with potential improvement of farm economics (Dochi, 2019; Alkan *et al.*, 2020).

Multiple studies have been conducted to elucidate factors affecting pregnancy establishment in embryo recipient heifers and cows after transfer of in vivo or in vitro produced embryos (Block *et al.*, 2009). Embryo quality grade was demonstrated to have significant effects on pregnancy rates (Van Soom *et al.*, 2001; Stroud and Hasler, 2006). Embryos of the highest grade were associated with significantly higher pregnancy rates than embryos of lower grades (Stringfellow, 2010; Alkan *et al.*, 2020). According to the study conducted by Alkan *et*

al., (2020), code 1 embryo (excellent or good) resulted in higher (P<0.05) pregnancy rates, when compared to code 2 embryo (Fair) after transfer into recipient beef heifers. Another factor to consider is interaction between the embryo, the uterine environment and the corpus luteum (Mann *et al.,* 1995). Cavities of corpus luteum are found in 40-80% of the estrous cycles of cows and heifers treated with progesterone or prostaglandin, and progesterone concentrations are not influenced by the presence of the cavity (Spell *et al.,* 2001; Marques *et al.,* 2002). However, the use of corpus luteum with cavity has generated controversy (Grygar *et al.,* 1997; Marques *et al.,* 2002; Looney *et al.,* 2006 and Siqueira *et al.,* 2009; Alkan *et al.,* 2020). Pregnancy rate in embryo recipient cows was not affected by the type of corpus luteum (compact versus cavitory) or circulating progesterone concentrations (Nogueira *et al.,* 2012).

Other factors related to recipient heifers – affecting establishment of pregnancy after transfer of in vivo produced embryos - include, but not limited to, heifer age; body weight and body measurement as height (Hasler, 2014). Some studies have found lower conception rate in rapidly growing heifers (Van Amburgh *et al.,* 1998). While others found similar conception between heifer groups with different daily gains (Hoffman *et al.,* 1996). A recent study by Senturklu *et al.,* (2015) showed that raising of prepubertal Holstein heifers to achieve higher average daily gain did not improve their conception, when compared to those raised to achieve lower daily gains. The age of heifers at first insemination did not affect fertility and conception, although differences were observed during the subsequent calving interval (Sakaguchi *et al.,* 2005). Thus, contradiction still dominates the effects of heifer daily gain, and consequently body weight, on establishment of pregnancy.

In this regard, the current work was conducted to study the effects of various recipient factors as age, weight, height and average daily gain of heifer, type of corpus luteum, type of heat and level of progesterone as well as effect of embryo quality grade, on PR/ET in embryo recipient Holstein heifers receiving fresh in vivo produced embryos.

MATERIALS AND METHODS

Animals

The current study was carried out on ninety-five recipient Holstein heifers in a commercial embryo transfer unit at a well-managed private Holstein herd (Sapphire Dairies, Lahor-Punjab, Pakistan). The experiment was carried out during the period from (January to February, 2018) during which the average daily temperature was 15 °C and the average relative humidity was 80 degrees. All animals were proven free from any infectious diseases and were routinely vaccinated against (FMD, BVD, IBR, Brucella, BEF, Clostridia and Pasteurella).

The selection of recipient heifers was based on their phenotypic characters, acceptable age from 13 to 21 months, with average 16.8 months, acceptable body weight, from 305 to 378 Kg at time of transfer. By average 327 kg also, the height of recipient heifers varied between 122 and 135 cm. with average 129 cm and all heifer have daily gain varied from 0.4 kg to 0.75 kg with average 0.6 kg per day as in (Table 1) confirmed puberty (presence of CL on the ovary or expression of heat signs) and free from infectious diseases or other health problems. Recipient variables (age, body weight, body height, average daily gain, grade of transferred embryo, type of corpus luteum and type of estrus) were retrieved from the automatic farm records (dairy comp DC305 management system).

Animals were fed a totally mixed ration which was constructed to meet or exceed the nutrient requirements of dairy cattle according to the NRC recommendations (NRC, 2001)

Recipient scanning

Recipient heifers were scanned four times by linear transducer Portable Ultrasonic Diagnostic System (SonoScape A5 Co. Ltd., China) provided with trans-rectal linear transducer (5 MHZ) for rectal scanning. The 1st scanning was carried out to avoid any congenital or pathological reproductive problems and to ensure normal size genitalia. The 2nd scanning was done during estrus to ensure and estimate heat onset. The 3rd scanning was carried out one day before transfer to identify the site of corpus luteum. The 4th scanning was carried out 30 days after transfer of embryos to diagnose pregnancy (Kasimanickam *et al.,* 2018).

Estrus synchronization

Recipient heifers were synchronized by single injection of PGF2α (500 µg Cloprostenol, ESTRUMATE "250µg/ml" MSD, USA), at

the same day of injection of PGF2α in donor cow to obtain the heat date of recipient heifers the same to the insemination date of donor cow.

The heifers which came in heat 24 hrs before or after donors AI date were kept for transferring of extra embryos. Some heifers come in heat without injection of PGF2α and we compare between them and induced heat heifers

Embryo loading

Embryos were graded according to International embryo transfer society criteria (IETS, 2010). Embryos were loaded in the straw as method described previously by (Dochi, 2019) and were transferred to recipient as method done by (Kasimanickam *et al.*, 2019).

Embryo transfer processing

The ovaries of recipient heifers were examined using ultrasonography to detect the type of the corpus luteum (compacted or Cavitory) one day prior to embryo transfer. Marking the side of the CL on the corresponding Gluteal region of the recipient animal with color marker for easy identifying and less handling of genitalia during the time of transfer. Posterior epidural anesthesia was used few minutes before transfer to recipient to avoid rectal resistance (5-7 ml of lidocaine). Complete hygienic measurement was used to keep vulva clean and dry before ET gun insertion. Additionally, the grade of transferred embryo was recorded for each recipient heifer Embryos were transferred non-surgically at day seven after estrus to synchronous recipient Holstein heifers through the recto vaginal technique into the anterior third of the horn through the cervix ipsilateral to the ovary containing corpus luteum

Pregnancy diagnosis

Pregnancy diagnosis in recipient heifers was carried out at 30 days after transfer using ultrasonographic portable device equipped with a 7.5 MHz linear rectal probe SonoScape A5. Confirmatory findings of pregnancy were the embryo and the surrounding amniotic membrane; embryonic heart beats and clear hypo-echoic fluids surrounding the embryo (Kasimanickam *et al.*, 2018).

Blood sampling

Each recipient was sampled once at time of embryo transfer from tail vein and blood samples were obtained from each recipient. The obtained samples were centrifuged at 3000 rpm for 15 minutes for separation of serum which was stored at – 80 degrees till analysis for progesterone (P4)

Progesterone assay:

Serum progesterone levels were assayed by Sandwich ELISA Quantitative micro-well technique using kits from Bioneovan. Co., Ltd. (China) and following manufacture instructions (Kasimanickam *et al.*, 2018).

Statistical analysis

Pregnancy rate per embryo transfer (PR/ET) was examined between different classifications using the chi square test. Chi square testing was employed to detect the differences in proportions between two or more of the examined variables. A p value <0.05 was considered significant, while a p value from above 0.05 to 0.1 was considered a statistical tendency (SPSS, 2007).

RESULTS

The recipients with different age had no significant differences ($P>0.05$) in the pregnancy rate per embryo transfer and the PR/ET was (64.44% and 64%) for younger recipients (less than 16.8 month) and older one (more than 16.8 month), respectively (Table 2) and (Fig.1). Concerning body weight, the body weight of recipient had no significant differences ($P>0.05$) in the pregnancy rate per embryo transfer and PR/ET has been recorded in heavier recipient heifers (more than 327 kg) in comparison to recipients having lower average body weights (less than 327 kg) (71.43% vs 53.85%), respectively (Table 3) and (Fig.1). Additionally, heifers having higher than average body height (more than 129 cm) achieved numerically higher PR/ET (67.93%), nearly 6 points higher than recipients with lower (less than 129 cm) than average body height (59.53%) (Table 4) and (Fig.1). Also, the average daily gain had no significant difference in PR/ET of recipient (71.34, 56.52 %) for higher average daily gain (more than 0.6 kg per day) and low average daily gains (less than 0.6 kg per day), respectively (Table 5) and (Fig.1).

Scanning of recipient revealed that there was no significant difference in PR/ET between recipients with cavitory CL (62.85%) and those with solid CL (65%) on day of embryo transfer (Fig.2) and (Fig.3).

Concerning the type of estrus, heifers with induced estrus achieved significant difference

(P<0.05) in PR/ET (72.73%), compared to those with spontaneous estrus (35.72%) (Table 6) and (Fig.4). In addition, the recipient heifers with high circulating levels (above 6.6 ng/ml) of progesterone at embryo transfer achieved higher (P<0.05) PR/ET when compared to those with low circulating levels (less than 6.6 ng/ml) (79.5% vs 55.4%, respectively). (Fig. 5)

Concerning grades of transferable embryos, PR/ET was higher in recipients receiving first grade embryo (79.17%), compared to 48.94% in recipients receiving second and third grade embryos (Fig.6) and (Fig.7).

Table 1): Age (month), body weight (kg) and body height (cm) measurements of recipient Holstein heifers (n=95).

Item	Minimum	Maximum	Mean ±SEM
Recipient age	13	21	16.8±0.15
Recipient body weight	305	378	327.15±1.49
Recipient height	122	135	129.04±0.26
Recipient daily gain	0.4	0.75	0.61

Table 2): Effect of age (month) of recipient on pregnancy rate per embryo transfer in recipient Holstein heifers (average age 16.8 month).

Items	Above average recipient age	Below average recipient age
First Grade Embryo transfers (n)	26	22
Second Grade Embryo and Third Grade Embryo transfers (n)	24	23
Transfers (n)	50	45
Pregnant (n)	32	29
PR/ET (%)	64.00	64.44

Table 3): Effect of body weight (kg) on pregnancy rate per embryo transfer in recipient Holstein heifers (average body weight 327 kg).

Items	Above average body weight	Below average body weight	P value
First Grade Embryo transfers (n)	28	20	NA
Second Grade Embryo and Third Grade Embryo transfers (n)	28	19	NA
Transfers (n)	56	39	NA
Pregnant (n)	40	21	NA
PR/ET (%)	71.43	53.85	0.06

Table 4): Effect of body height (cm) on pregnancy rate per embryo transfer (PR/ET) in recipient Holstein heifers (average height 129 cm).

Items	Above average body height	Below average body height	P value
First Grade Embryo transfers (n)	32	17	NA
Second Grade Embryo and Third Grade Embryo transfers (n)	21	25	NA
Transfers (n)	53	42	NA
Pregnant (n)	36	25	NA
PR/ET (%)	67.93	59.53	0.26

Table 5): Effect of daily gain on pregnancy rate per embryo transfer (PR/ET) in recipient Holstein heifers (Average daily gain 0.6 kg per day).

Items	High average daily gain	Low average daily gain	P value
First Grade Embryo transfers (n)	25	23	NA
Second Grade Embryo and Third Grade Embryo transfers (n)	24	23	NA
Transfers (n)	49	46	NA
Pregnant (n)	35	26	NA
PR/ET (%)	71.43	56.52	0.39

Table 6): Effects of type of recipient heat (spontaneous vs induced) on PR/ET in recipient Holstein heifers

Recipient heat	Spontaneous	Induced	P value
First Grade Embryo transfers (n)	6	15	NA
Second Grade Embryo and Third Grade Embryo transfers (n)	8	18	NA
Transfers (n)	14	33	NA
Pregnancy (n)	5	24	NA
PR/ET (%)	35.72	72.73	0.02

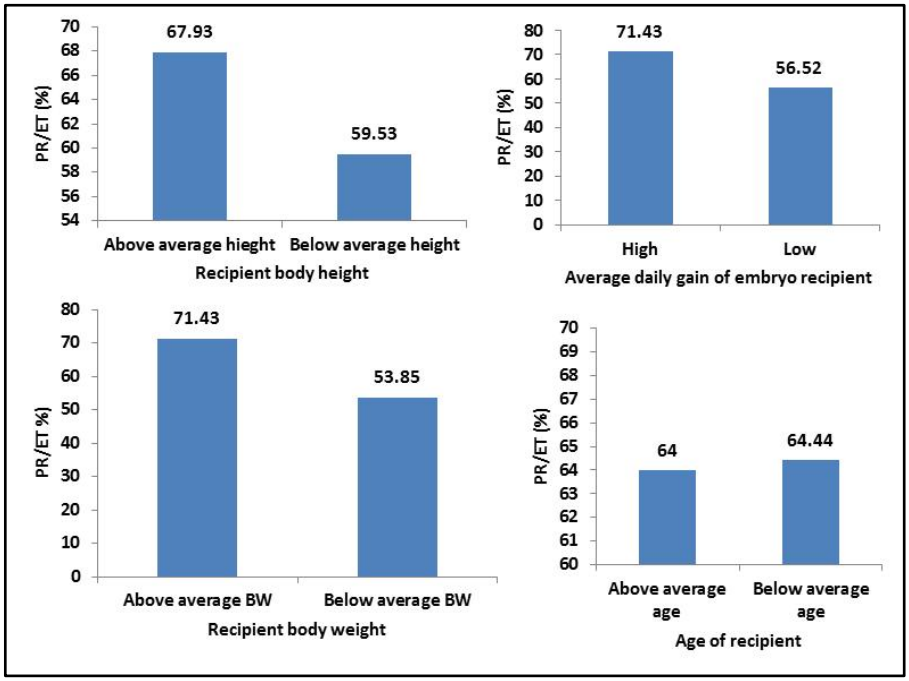

Figure 1: Variation of PR/ET between different recipient categories (age, body weight, body height, and average daily gain) (P>0.05).

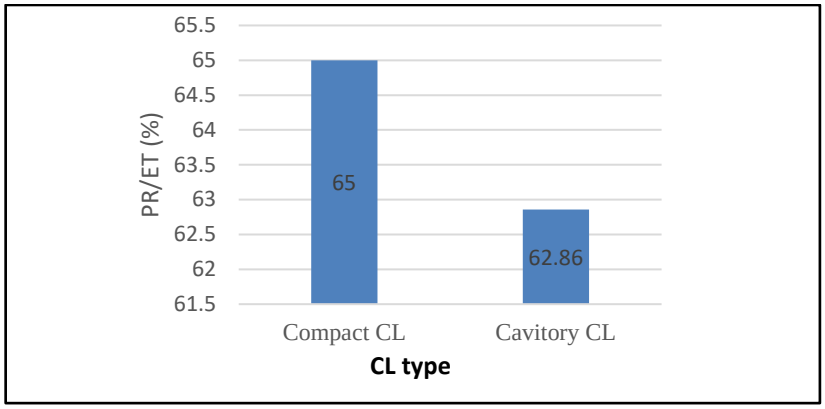

Figure 2: Effect of recipient CL type in Holstein heifers (compact vs cavitory) on pregnancy establishment after embryo transfer (P>0.05).

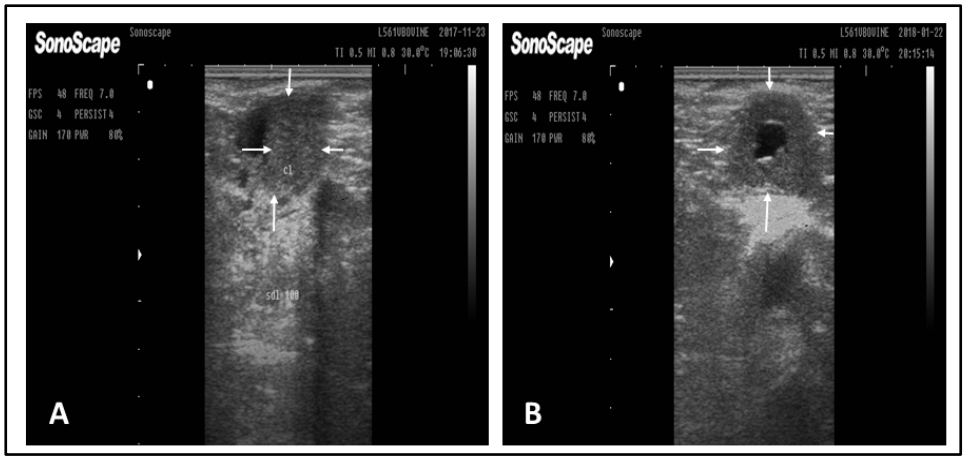

Figure 3: Ultrasonographic image of recipient Holstein heifer's ovaries showing (A) compact corpus luteum and (B) cavitory corpus luteum (arrow).

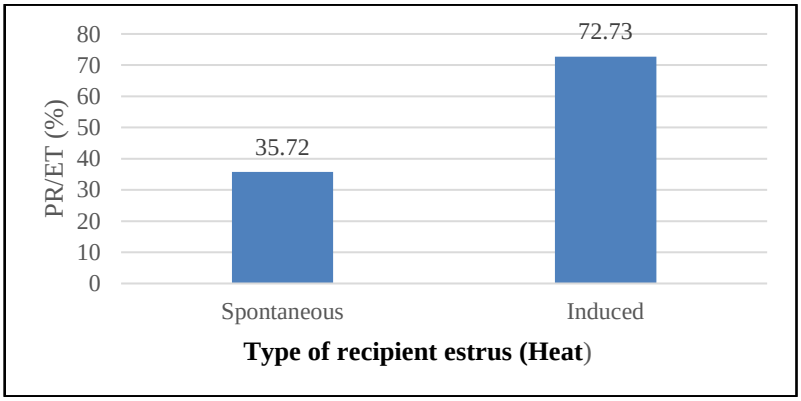

Figure 4: Effect of type of recipient heat (spontaneous vs induced) on PR/ET in Holstein heifers (P<0.05).

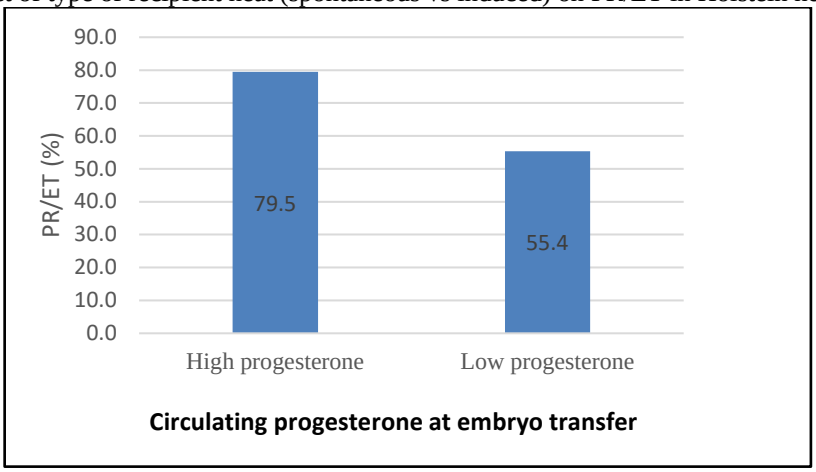

Figure 5: Effect of circulating progesterone on day of embryo transfer on PR/ET in recipient Holstein heifers (P<0.05).

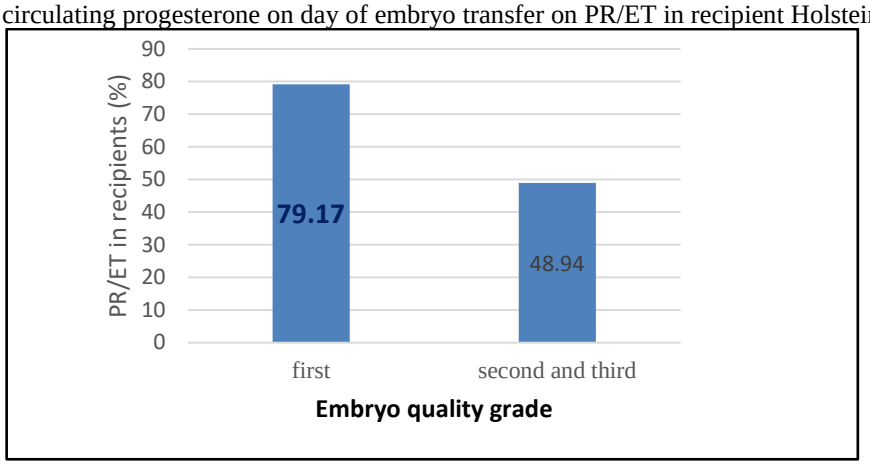

Figure 6: Effect of quality grades of transferred embryos on PR/ET in recipient Holstein heifers (P<0.05).

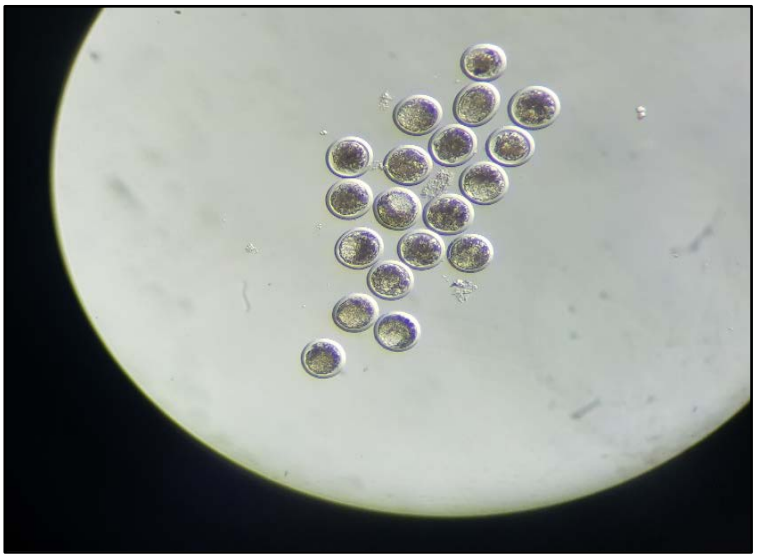

Figure7: Transferred in vivo produced embryos under stereo-microscope (X60) in culture dish.

DISCUSSION

The role of recipients of in vivo or in vitro transferred embryos in the establishment of pregnancy after embryo transfer and how several recipient factors can affect conception rate to embryo transfer have been reviewed by Hasler (2014). In addition, recipient heifer temperament and its effect on establishment of pregnancy after embryo transfer has been studied by Kasimanicam *et al.*, (2018).

The study was revealed that factors as recipient age, body weight, body height and average daily gain did not impact the risk or pregnancy establishment after embryo transfer. In addition, the source of produced embryos, in other words the scheme of superovulation of donor cows, did not influence PR/ET in recipient heifers. Further, PR/ET did not vary among recipients with cavitory or solid CL. On the other hand, recipient circulating levels of progesterone at embryo transfer, quality grade of transferred embryo and recipient heat type were associated with significant variations of PR/ET

No differences were observed between different age categories in pregnancy per embryo transfer in the present work. Recipient heifers older than the average age achieved nearly the same PR/ET as that observed in recipients younger than the average age. While, Boligon and Albuquerque (2011) concluded that attainment of earlier puberty, which can be achieved by management of nutritional allowances during prepubertal heifer stages can affect pregnancy rate to first AI

Heavier heifers tended to achieve greater pregnancy rates (P=0.06), than those with lighter body weight (average body weights at embryo transfer 327.14 Kg). Lopez *et al.*, (2018) reported higher first service pregnancy rates in heifers with high weights even at weaning

Findings of the current work revealed that numerically higher PR/ET was established in heifers with height higher than the average body height at embryo transfer. In spite of that, these differences were not statistically different. Senturklu *et al.*, (2015) reported that small frame and large frame heifers were able to achieve similar pregnancy rates and growth performances when fed on three phases prior to the planned breeding season.

In the present study, PR/ET did not differ significantly between recipient heifers with high or low average daily gain. Similar results were documented by (Lopez *et al.*, 2018). In the present study, CL type did not affect PR/ET in recipients. Nogueira *et al.*, (2012) mentioned the same obtained results, and reported that pregnancy rate in embryo recipient cows was not affected by the type of corpus luteum (compact versus cavitory). The same authors reported that the cavity of CL did not alter circulating progesterone levels in recipient cows. Nevertheless, other research papers reported that increase in the concentration of P4 in accordance with the corpus luteum increase in crossbred recipients and according to Binelli *et al.*, (2001), larger corpus luteum in recipients may increase pregnancy rates in embryo transfer programs. On the other hand, Alkan *et al.*, (2020)

reported that cavity-containing corpora lutea resulted in higher pregnancy rates in heifers receiving fresh in vivo produced embryos. In addition, that, Szelenyi *et al.,* (2019) found that pregnancy losses in Holstein-Friesian cows examined by ultrasonography between days 29-42 after insemination were higher among cows with cavity-containing corpora lutea

The present investigation cleared that PR/ET was significantly higher in embryo recipient Holstein heifers in which estrus or heat was induced using PGF2 alpha, as compared to recipient heifers with spontaneous heat. In accordance with our results, in his review, Hasler (2014), documented that PR/ET was higher in recipient heifers with induced estrus after PGF2 alpha, as compared to those with natural estrus. Heifers are known to predominantly express two-wave estrous cycles in which the developing oocytes are exposed to greater levels of progesterone than reported in the two-wave cycle commonly observed in cows (Baruselli *et al.,* 2012). The longer the time of exposure to progesterone, the lower the developmental competence of the developing oocytes, however, it is not yet clear if this applies also to the developing embryo

Circulating progesterone levels at embryo transfer did influence PR/ET in recipient Holstein heifers in the current study. Impacts of circulating progesterone at time of insemination or at time of embryo transfer on pregnancy establishment have been studied previously (Niemann *et al.,* 1985; Gisert *et al.,* 1992; Nishigai *et al.,* 1998; Spell *et al.,* 2001). Nishigai *et al.,* (2000) found that high progesterone increased pregnancy rate in embryo which agreed with the obtained result in the current study. The effects were attributed to the possible role of progesterone on uterine environment (Gisert *et al.,* 1992).

In the present study, the result indicated that first grade embryo was associated with significantly higher PR/ET, when compared to second and third grade embryo classes. The obtained result agrees with that reported by Alkan *et al.,* (2020) reported higher PR/ET after transfer of code 1 embryo, in comparasion to code 2 embryo. In the same context, multiple studies have demonstrated that there are significant differences in pregnancy rates across different embryo quality grades (Van Soom *et al.,* 2001; Stroud and Hasler, 2006; Block *et al.,* 2009). In addition, embryo grade was identified as an important factor in determining pregnancy rate (Looney *et al.,* 1994). Embryos of the highest grade were associated with significantly higher pregnancy rates than embryos of lower grades.

Conclusion

In conclusion, several factors as age, body weight, body height and daily gain of recipient Holstein heifers were shown to have no effect on PR/ET while, circulating progesterone levels, recipient heat type and embryo quality grade had significantly impacted the rate of pregnancy establishment in embryo recipient Holstein heifers.

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Conflict of interest

The authors declare no conflict of interest.

REFERENCES

Alkan, H., Karasahin, T., Dursun, S., Satilmis, F., Erdem, H., Guler, M. 2020. Evaluation of the factors that affect the pregnancy rates during embryo transfer in beef heifers. Reprod. Domest. Anim., 55: 421-428.

Baruselli, P.S., Sa. M.A., Ferreira, R.M., Sales, J.N.S., Gimenes, L.U., Vieira, L.M., Mendanha, M.F., Bo, G.A. 2012. Manipulation of Follicle Development to Ensure Optimal Oocyte Quality and Conception Rates in Cattle. Reproduction in Domestic Animals 47:134-141.

Binelli, M., Thatcher, W.W., Mattos, R., Baruselli, P.S. 2001. Antiluteolytic strategies to improve fertility in cattle. Theriogenology, 56, 1451-1463.

Block, J., Bonilla, L., Hansen, P.J. 2009. Effect of addition of hyaluronan to embryo culture medium on survival of bovine embryos in vitro following vitrification and establishment of pregnancy after transfer to recipients. Theriogenology; 71:1063-1071.

Boligon, A.A., Albuquerque, L.G. 2011. Genetic parameters and relationships of heifer pregnancy and age at first calving with weight gain, yearling and mature

weight in Nelore cattle. Livestock Science, 143: 12-16.

Dochi, S. 2019. Direct transfer of frozen-thawed bovine embryos and application in cattle reproduction management. J. Reprod. Manag., 65(5): 389-396.

Farin, P.W., Britt, J.H., Shaw, D.W., Slenning, B.D. 1995. Agreement among evaluators of bovine embryos produced in vivo or in vitro. Theriogenology; 44:339-349.

Farin, P.W., Slenning, B.D., Britt, J.H. 1999. Estimates of pregnancy outcomes based on selection of bovine embryos produced in vivo or in vitro. Theriogenology; 52:659-670.

Gisert, R.D., Morgan, G.L., Short, E.C., Davy, M.D. 1992. Endocrine events associated with endometrial function and conceptus development in cattle. Reprod. Fertil. Deve.; 4: 301-305.

Grygar, I., Kudlac, E., Dolezel, R., Nedbalkova, J. 1997. Volume of luteal tissue and concentration of serum progesterone in cows bearing homogeneous corpus luteum or corpus luteum with cavity. Animal Reproduction Science, 49:77-82.

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 reminisces. Theriogenology, 81:152–169

Hoffman, P.C., Brehm, N.M., Price, S.G., Prill-Adams, A. 1996. Effect of accelerated postpubertal growth and early calving on lactating performance of primiparous Holstein heifers. Journal of Dairy Science, 79: 2024–2031.

IETS (International Embryo Transfer Society, 2010): In: Stringfellow DA, Givens MD, editors. Manual of the international embryo transfer society: a procedural guide and general information for the use of embryo transfer technology emphasizing sanitary precautions. Fourth ed. Champaign, IL: IETS.

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: 70-78.

Kasimanickam, R., Kasimanickam, V., Gold, J., Moore, D., Kastelic, J.P., Pyrdek, D., Ratzburg, K. 2019. Injectable or transdermal flunixin meglumine improves pregnancy rates in embryo transfer recipient beef cows without altering returns to estrus. Theriogenology, 140: 8-17.

Lindner, G.M., Wright, Jr. R.W. 1983. Bovine embryo morphology and evaluation. Theriogenology, 20:407-416.

Looney, C.R., Lindsey, B.R., Gonseth, C.L., Johnson, D.L. 1994. Commercial aspects of oocyte retrieval and in vitro fertilization (IVF) for embryo production in problem cows. Theriogenology, 41:67-72.

Looney, C.R., Nelson, J.S., Schneider, H.J. 2006. Improving fertility in beef cow recipients. Theriogenology, 65:201-209.

Lopez, E., Veliz, F.G., Carillo, E., De Santiago, A., Garcia, J., Mellado, M. 2018. Effect of body weight, weaning weight and preweaning weight gain on fertility of Holstein heifers under hot Mexican conditions. Slov. Vet. Res., 55(1): 35-42.

Mann, G.E., Lamming, G.E., Fray, M.D. 1995. Plasma oestradiol and progesterone during early pregnancy in the cow and the effects of treatment with buserelin. Anim. Reprod. Sci., 37: 121-131.

Marques, M.O., Arruda, R.P., Madureira, E.H. *et al.*, 2002. Effect of corpus luteum cavity on plasma progesterone concentration in Bos taurus x Bos indicus embryo recipient heifers. Revista Brasileira de Reprodução Animal, 26: 238-240.

Niemann, H., Sacher, B., Elsaesser, F. 1985. Pregnancy rates relative to recipient plasma progesterone levels on the day of frozen/thawed bovine embryo. Theriogenology, 23: 631-639.

Nishigai, M., Kamomae, H., Tanaka, T., Kaneda, Y. 1998. Pregnancy rate and blood progesterone concentrations on the previous day and the day of frozen embryo transfer in parous recipient cows of Japanese black. J. Reprod. Dev., 44: 413-419.

Nishigai, M., Kamomae, H., Tanaka, T., Kaneda, Y. 2000. The relationship of blood progesterone and estrogen concentrations on the day before and the day of frozen-thawed embryo transfer to

pregnancy rate in Japanese black beef cattle. J. Reprod. Dev., 46(4): 235-243.

Nogueira, M.G.F., Cardoso, G.S., Jonior, H.R.M., Dias, A.M., Itavo, L.C.V., Borges, J.C. 2012. Effect of breed and corpus lueum on pregnancy rate of bovine embryo recipients. Revist. Brazil. De Zootecn., 41(9): 2129-2133.

N.R.C 2001. Nutrient requirements of dairy cattle, 7th revised. Washington, DC, USA: National Academic Science.

Sakaguchi, M., Suzuki, T., Sasamoto, Y., Takahashi, Y., Nishiura, A., Aok, M. 2005. Effects of first breeding age on the production and reproduction of Holstein heifers up to the third lactation. Anim. Sci. J., 76: 419-426.

Senturklu, S., Landblom, D.G., Perry, G.A., Petry, T. 2015. Effect of heifer frame score on growth, fertility and economics. Asian Austr. J. Anim. Sci., 28(1): 69-78.

Siqueira, L.G.B., Torres, C.A.A., Souza, E.D., Monteiro, P.L.J., Viana, J.H.M. 2009. Pregnancy rates and corpus luteum–related factors affecting pregnancy establishment in bovine recipients synchronized for fixed-time embryo transfer. Theriogenology, 72: 949-958.

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): 287-297.

SPSS.2007 Statistical package for social sciences, user's guide for statistical analysis version 15, free software internet download.

Stringfellow, D.G.M.D. 2010. Manual of the International Embryo Transfer Society: A procedural guide and general information for the use of embryo transfer technology, emphasizing sanitary precautions. Champaign, IL: International Embryo Transfer Society;

Stroud, B., Hasler, J.F. 2006. Dissecting why superovulation and embryo transfer usually work on some farms but not on others. Theriogenology, 65:65-76.

Szelenyi, Z., Gyori, D., Boldizsar, S., Kovacs, L., Repasi, A., Molnar, L., Szenci, O. 2019. Pregnancy and stillbirth losses in dairy cows with singleton and twin pregnancies. Act. Vet. Hung., 67(1): 115-126.

Van Amburgh, M.E., Galton, D.M., Bauman, D.E., Everett, R.W., Fox, D.G., Chase, L.E., Erb, H.N. 1998. Effects of three prepubertal body growth rates on performance of Holstein heifers during first lactation. Journal of Dairy Science, 81: 527–538.

Van Soom, A., Vanroose, G., de Kruif ,A. 2001. Blastocyst evaluation by means of differential staining: a practical approach. Reprod Dom Anim., 36:29-35.