Effects of Human Chorionic Gonadotrophin, Flunixin Meglumin, Lidocane on Pregnancy Rate with Hanwoo IVF Embryo Transfer

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ABSTRACT

This study was carried out to confirm the effects of luteotrophin, human chorionic gonadotrophin (hCG), and an anti-luteolytic agent, flunixin meglumin (FM), on pregnancy rates in Hanwoo with *in vitro* produced (IVP) embryo transfers (ET), and to research the effects on the estrus cycle. Treatments included hCG and FM administration 3~10 minutes prior to ET. Also, pregnancy rates were compared with lidocane treatment and FM treatment prior to ET. The results are shown below.

- 30-day pregnancy rate was 76.7% in the hCG-treated group and 75.7% in the FM-treated group. Both rates were higher than the 70% rate for the control group.
- 42-day pregnancy rate was 76.7% in the FM-treated group. This was higher than 66.7% recorded for both the hCG-treated and control groups.
- The pregnancy rate of the hCG-treated group was high at Day 30 (76.7%) but low at Day 40 (66.7%), and there were no differences from the FM-treated and control groups.
- The recurrent estrus rate of infertile individuals at 2 weeks after ET was 36.4% in the hCG-treated group, under 71.4% in the FM-treated group and 80.0% in the control group.
- The non-pregnancy rate of individuals without recurrent estrus was 18.2% in the hCG-treated group, which was higher than the 0% rate in both the FM-treated and control groups.
- The pregnancy rates were higher in the FM-treated group than the Lidocane-treated group with 72.3% versus 67.5% in the heifers and 48.9% versus 43.6% in the cows.

From the above results, the FM treatment proved more effective than the hCG treatment and no treatment whatsoever in increasing pregnancy rates after ET. In addition, hCG treatment was shown to be undesirable due to the deviations it caused in the reproductive physiology of the hCG-treated recipients. Therefore, in our study, the FM treatment resulted in a higher pregnancy rate than either lidocaine treatment or no-treatment in the trials of ET.

(Key words : hCG, FM, lidocaine, *in vitro* fertilization, embryo transfer)

INTRODUCTION

The roles and expectations for embryo transfer can be accomplished by using the best method to greatly proliferate females with excellent genetic abilities. In the field, the pregnancy rate of ET with *in vitro* fertilized (IVF) embryos is low compared to artificial insemination. Pregnancy rates accompanying IVF will be maximized if the following three conditions are all satisfied: high quality embryos are used, accurate techniques for the fine control over the transfer to the optimal site without causing any damage to the endometrium are employed, and that a recipient with a good reproductive environment for the implantation of the embryo is used and the pregnancy is maintained. Also, an increase in pregnancy rates by using external corpus luteum (CL)-affecting substances may considerably contribute to the roles and expected results of ET.

The relationship between the maternal body and the embryo is not fully understood (Wathes *et al.*, 1998, Mann *et al.*, 1999; Thatcher *et al.*, 2001; Wolf *et al.*, 2003). It has been reported that progesterone (P4) regulates the luteolytic signals (Mann *et al,*. 1995; Bogacki *et al.*, 2002) and the growth and development of a fetus (Garret *et al.*, 1988; Kerbler *et al.*, 1997). It has been reported that P4, which signi-

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ficantly influences the fetus, shows high plasma concentrations at 5~7 days after estrus (Butler *et al.*, 1996; Dunne *et al.*, 1999; Starbuck, 1999; Kenny *et al.*, 2001), but not in ET due to the influence of transferred embryos (Smith *et al.*, 1996; Spell *et al.*, 2001; Chagas *et al.*, 2002). This is in line with the view that the embryo in ET delays the environmental possibility of the CL (Chagas & Lopes, 2005). Certain studies have reported that hCG administration leads to the formation of the accessory CL and maintains a high P4 concentration that would enhance the survival rate of the fetus and the permanence of the CL (Helmer & Britt, 1986; Price & Webb, 1989; Walton *et al.*, 1990; Sianangama and Rajamahendran, 1992; Schmitt *et al.*, 1996; Diaz *et al.*, 1998; Santos *et al.*, 2001; Nishigai *et al.*, 2002). Thus, the reproductive organs of a maternal body to which embryos are transferred would be expected to be a good environment for its implantation and maintenance. It is reasonable to assume that the plasma CL concentration surrounding the reproductive organs affects the survival of the fetus compared to that of the distal parts from the uterus. One study reported that pregnancy is maintained normally even if there are low plasma P4 concentrations in the distal regions of the reproductive organs (Hasler *et al.*, 1980; Stubbings & Walton, 1986; Smith *et al.*, 1996; Spell *et al.*, 2001; Chagas *et al.*, 2002). Shemesh *et al.* (1979) reported that blastocysts cultured for 13~16 days secrete P4 into a culture solution. From the results of these studies, a study investigating the effect of hCG treatment on fertility rates and pregnancy maintenance of recipients after embryo transfer would be an interesting proposition.

During embryo transfer, an operator holds and lifts the uterus and maneuvers the catheter so as not to touch the uterine wall, as this could damage to endometrium, during the process of placing embryo in the deep site of the uterine horn without damaging the endometrium. The stimulation to the uterus during this process results in the secretion of prostaglandin$F_{2\alpha}$ ($PGF_{2\alpha}$) from the endometrium (Ferguson, 1941). The secretion of $PGF_{2\alpha}$ directly affects the survival of the embryo (Schrick *et al.*, 2003; Seals *et al.*, 1998). By administrating FM to inhibit the production of $PGF_{2\alpha}$, which is fatal to the fetus, Percell *et al.* (2005) and Schrick *et al.* (2001) reported improved pregnancy rates.

The main objectives of this study were to find methods that are able to increase pregnancy rates following ET. Thus, this study compared pregnancy rates and sought to establish a method for increasing final pregnancy rate by increasing plasma P4 concentration with hCG treatment and inhibiting the production and secretion of $PGF_{2\alpha}$ by FM treatment. Also, on the expectation of increased pregnancy rates, we compared pregnancy rates following FM and Lidocane treatment in order to establish the clinical viability of our method.

MATERIALS AND METHODS

1. *In Vitro* Production of Embryos

Follicles with a diameter of 2~5 mm were aspirated from the ovaries of Korean native cows derived from a slaughterhouse and cumulus-oocyte complexes (COCs) surrounded by more than one layer of cumulus cells with distinct cytoplasm were selected. These were then cultured for IVF after properly removing the cumulus cells in Medium199 with 3% FBS and 1 μl/ml Gentamycin. Immature COCs were matured in Medium199 solution containing 5% FBS, 30 ng EGF/ml, and 1 μl/ml Gentamycin for 20~22 h at 5% CO_2, 39℃, and maximized humidity in air. Basic BO solution (Brackett *et al.*, 1975) was modified (m-BO) and used as the fertilization media composed of 113.5 mM NaCl, 4.02 mM KCl, 2.25 mM $CaCl_2$, 0.83 mM Na_2HPO_4, 0.52 mM $MgCl_2 \cdot 6H_2O$, 25.0 mM $NaHCO_3$, 1.25 mM Na-pyruvate, 5.0 mM glucose, 12.5 mM HEPEs, 5 mg BSA (Sigma A8806)/ml, and 1 μl/ml gentamycin. The m-BO solution to which heparin 20 g/ml and 10 mM caffeine-sodium benzoate added was used for COCs washing and semen treatment. Then, COCs were washed three times in m-BO and then placed in the same m-BO drop (COCs number × 2 μl) covered with mineral oil for IVF. Frozen Hanwoo semen was thawed at 35℃ for 30 seconds, centrifuged twice at 800×g for 8 minutes and placed in the fertilization solution (m-BO) drop at 2×10^6/ml concentration. After 8 hours of fertilization, the COCs were rinsed three times in Medium199 containing 5% FBS and 1 μl/ml Gentamycin and then transferred in the mature media replaced by 50% of this new solution and cultured for 48 hours. Zygotes that grew by more than four cells were cultured in a culture medium of Medium199 containing 0.8% BSA (Sigma A8806) and 1 μl/ml Gentamycin for 9 days. The culture medium was replaced by 50% every 48 hours.

2. Synchronization

Estrus was artificially induced in 13~15 month old heifers with BCS 2~3, which were bred as part of the same group on a farm. The gonadotrophin releasing hormone (GnRH) was

injected on the first day of induced estrus and controlled internal drug releasing device (CIDR, CIDR^{TM}, New Zealand) was simultaneously inserted in the vagina, which was then removed after seven days. When removing the CIDR, $PGF_{2\alpha}$ (Lutalyse, Upjohn, Belgium) was injected followed by the GnRH after confirming estrus (48 hours after $PGF_{2\alpha}$ injection).

3. Treatments

Experiment 1) The hCG treatment group received 1,500 IU hCG (Choluron, Holland) and the FM treatment group received 250mg Flunixin Meglumine (Fluximin, Bomac New Zealand) by intramuscular injection prior to ET. The control group was left untreated.

Experiment 2) The FM treatment group was treated as detailed above and the Lidocaine treatment group received a 5 ml of 0.2% lidocaine injection for epidural anesthesia just before ET.

4. Embryo Transfer

Only blastocysts at 7~8 days of IVF were used for embryo transfer. The blastocysts were non-surgically transferred to the deep site of the uterine horn, where the CL is present in synchronized recipients at 7~8 days, after identifying estrus (48 hours after $PGF_{2\alpha}$ administration). It took almost one hour to arrive at the location and another 3~8 minutes for actual ET to occur. One blastocyst was transferred to heifers and two blastocysts of the same developmental stage were transferred to cows. All ET were performed by the same technician using the same tools and method.

5. Pregnancy Diagnosis

Pregnancy was diagnosed at Day 23 and Day 35 after ET by transrectal ultrasonography (Tringa Linear, Esaote Piemedical). Pregnancy was also determined on the basis (Kastelic *et al.*, 1991) of the uterine cavity and the presence of uterine luminal fluid, an embryonic vesicle, fetus and heartbeat, and palpations of the middle uterine artery.

6. Statistics

A total of 100 dairy heifers were used for estrus induction, which was induced in 20 cows at a time, and randomly assigned to each treatment group. This was repeated five times. Seven cows in the synchronized group that showed estrus at transfer day were excluded from the experiment.

Hanwoo IVP blastocysts were transferred in 220 heifers and 192 cows to compare the pregnancy rates with the FM treatment group and lidocaine treatment group. This was repeated three times, and recipients were randomly assigned to one of the two groups at the time of transfer.

The significance test in *t*-test was conducted to analyze the data.

RESULTS AND DISCUSSION

Experiment 1) The results for Experiment 1 following FM treatment or hCG treatment on dairy heifers are shown in Table 1.

From the above results, the pregnancy rate following FM-treatment at Day 42 pregnancy diagnosis (35 days after ET) showed the highest pregnancy rate at 76.7% ($p<0.05$). The hCG treated group and control group showed 66.7% and 66.7%, respectively (not significantly different at $P>0.5$). These findings agree with those of Schrick *et al.* (2001) that the present study recorded a 12.7% higher pregnancy rate in the FM-treated group. Our findings are also in agreement with Pur-

Table 1. Comparison of pregnancy rates following treatments of hCG and FM prior to ET

Treatments	No. of heifers transfered	No. of pregnant hifers at day 23 post-ET (%)	No. of pregnant hifers at day 35 post-ET (%)	No. of heifers showing early fetaldeath
hCG	33	25 (75.7%)[a]	22 (66.7%)[a]	3 (9.1%)[a]
FM	30	23 (76.7%)[a]	23 (76.7%)[b]	0 (0.0%)[b]
Control	30	21 (70.0%)[b]	20 (66.7%)[a]	1 (3.3%)[b]

[a~c] Values with different superscripts are different ($p<0.05$). Hifers were assigned to one of three treatments and experiments were repeated five times; FM-treated hifers were given 250 mg of flunixin meglumin (FM) at the time of ET; HCG-treated hifers were given 1,500 IU of hCG at the time of ET; Control hifers received no treatments; Pregnant diagnosis were carried out at Day 23 and Day 35 post-ET by ultrasonography.

cell *et al.* (2005) in which 65.0% and 74.7% pregnancy rates were observed. That is, FM treatment inhibited the production and secretion of $PGF_{2\alpha}$ due to uterus stimulation. Also, there was no difference between the hCG treatment group (66.7%) and the control group (66.7%), which contradicts the results of Nichigai *et al.* (2002) and Santos *et al.* (2001) who reported significant increases in pregnancy rates in hCG-treated groups. However, our findings are in agreement with those of Chagas & Lopes (2005) and Schmitt *et al.* (1996) who were unable to show differences in pregnancy rates. Our findings showed that FM treatment increased pregnancy rates, and thus might be deemed a viable method for increasing pregnancy rate after ET. We believe that hCG- treatment which was expected to enhance pregnancy rates in the implantation and survival of transferred embryos does not actually offer an optimal environment to the transferred embryo, i.e. it did not provide an increase in pregnancy rates as expected.

Pregnancy was diagnosed at Day 30 (23 days after embryo transfer) and Day 42 (35 days after embryo transfer) using an ultrasonic imaging diagnostor (ultrasonography). Pregnancy diagnosis (Table 1) showed that the pregnancy maintenance rate at Day 30 in the hCG treatment group and FM treatment group was high at 75.7% and 76.7%, respectively, and low in the control group at 70.0%. These results were similar to those of Chagas and Lopes (2004) in which the pregnancy rate in the hCG-treated group was 53.5% compared to the 38.1% of the control group and those of Nichigai (2003) in which a higher pregnancy rate was recorded at Day 28 in the hCG-treated group compared to the control group. Our findings are also similar to the results of Purcell *et al.* (2005) in which the FM-treated group showed a 12.7% increased pregnancy rate compared to the control group. However, the test results show that the treatment group and the control group showed similar levels of pregnancy rates with 63.7% and 66.7% respectively on Day 42 pregnancy diagnosis ($P>0.5$), which corresponds to the test results of the hCG treatment and non-treatment study by Chagas and Lopes (2004) and Schmitt *et al.* (1996). The pregnancy rate of the treatment group was higher than the control group on Day 28 pregnancy diagnosis but the two groups showed similar figures on Day 42 diagnosis. Three heads (9.1%) among Day 30 pregnancy-confirmed recipients in the hCG treatment group were diagnosed as infertile at Day 42 diagnosis due to the early death of the fetus ($p<0.05$), which suggests that the hCG treatment does not have a good influence on pregnancy maintenance.

This suggests that hCG treatment is not positively effective in the maintenance of pregnancy ($p<0.05$). The number of early deaths of fetuses was 0 (0.0%) and 1 (3.3%) in the FM group and the control group, respectively, and pregnancy was maintained without significant differences between these groups ($p>0.5$). This result is at odds with most of the other studies that showed increased plasma P4 concentration due to hCG treatment which positively affected the maintenance of pregnancy. Therefore, our results show that there are undesirable effects with hCG treatment, and showed that FM treatment prior to ET may be considered a more viable method for increasing pregnancy rates and maintaining pregnancy than hCG treatment or no treatment whatsoever. Thus, more studies are needed to determine the other effects on pregnancy besides hCG treatment.

The recurrence rate of estrus was also compared between the three groups and the results are shown in Table 2.

As shown in Table 2, the estrus recurrence rate was different in the hCG-treated group compared to the FM-treated group and the control group. In the case of cows with a

Table 2. Effects of hCG and FM treatments on recurrent of estrus

Treatments	No. of non-pregant heipers	No. of hifers return to the estrus at 2 weeks (%)	No. of hifers return to the estrus at 17days to 5weeks (%)	No. of not-estrus hifers in non-pregnants
hCG	11	4 (36.4%)[a]	7 (63.7%)[a]	2 (18.2%)[a]
FM	7	5 (71.4%)[b]	2 (28.6%)[b]	0 (0.0%)[b]
Control	10	8 (80.0%)[b]	2 (20.0%)[b]	0 (0.0%)[b]

[a,b] Values with different superscripts are different ($p<0.05$). Hifers were assigned to one of three treatments and experiments were repeated five times; FM-treated hifers were given 250 mg of flunixin meglumin (FM) at the time of ET; hCG-treated hifers were given 1,500 IU of hCG at the time of ET; Control hifers received no treatments; Pregnant diagnosis were carried out at Day 23 and Day 35 post-ET with ultrasonography.

three week reproductive cycle, non-pregnant animals should be in the recurrent estrous condition at two weeks after ET, but the number of animals with a normal recurrent estrous cycle was 4 (36.4%) in the hCG-treated group, 5 (71.4%) in FM-treated group, and 10 (80.0%) in the control group. The number of animals that were out of cycle and recurred to estrus was as high as 7 (63.7%) in the hCG-treated group compared to 2 (28.6%) in the FM-treated group and 2 (20.0%) in control group. The individuals that showed recurrent estrus after the expected recurrence date were sporadically present in the hCG-treated group as shown in Fig. 1.

Also, there was no animal without recurrent estrus until pregnancy diagnosis at 23 days following transfer in the FM treatment group and control group, whereas 2 (18.2%) were present in the hCG-treated group. These infertile and non-recurrent estrous individuals had permanent CL of considerable sizes, which was considered to be due to hCG treatment. It was reported that hCG treatment has been shown to delay the estrus period (Nishigai, 2003) and delay luteolysis (Chagas and Lopes, 2004; Schmitt *et al.*, 1996). Shagas & Lopes (2004) also reported that enlarged CL due to hCG treatment might degrade after 21 days, which considers that luteolysis is delayed compared to the normal cycle, which could result in delayed estrus. Our findings have shown what appear to be the twisted physiological phenomenon of non-pregnant individuals naturally showing recurrent estrus because of increased plasma P4 concentration, while other previously mentioned results showed that hCG treatment induced the formation of the accessory CL, increased progesterone (P4) secretion from the accessory CL and spontaneous CL, and then increased P4 concentration, which had a positive effect on the implantation and maintenance of pregnancy. As a result, hCG treatment showed a higher pregnancy expectation and improved pregnancy rates at Day 28 pregnancy diagnosis. However, pregnancy rates were shown to be the same at Day 42 pregnancy and the recurrent estrus of non-pregnant individuals was delayed resulting in a longer non-pregnant periods. In the FM-treated group, the recurrent estrus of non-pregnant individuals naturally occurred along with an increased pregnancy rate, resulting in improved reproductive efficiency and increased farmhouse income. Therefore, FM treatment may be considered a desirable method for ET without a not-twisted recurrent estrous in the infertile individuals than that which occurs with hCG treatment.

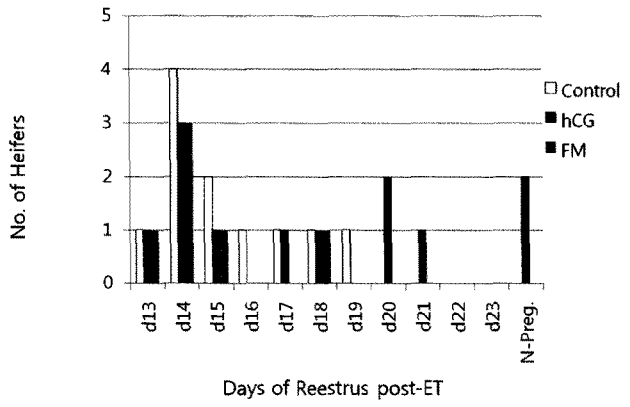

Fig. 1. Distribution of reestrus following treatments.

Experiment 2) Pregnancy rates were compared between the lidocaine treatment group and the FM treatment group, composed of heifers and cows, under the assumption that lidocaine, a local anesthetic, might improve pregnancy rate after ET. The results are shown in Table 3.

Lidocaine was used as a method for reducing the pain or unpleasant stimulation from a process of ET and stabilizing recipients. When the embryo was transferred after epidural anesthesia the pregnancy rate was 67.5%, which was lower than the 72.3% as seen with FM treatment. The reason for this may be that epidural anesthesia improves the stability of recipients and increases the ease by which an operator can handle a catheter during ET whilst having no inhibitory effect on $PGF_{2\alpha}$ production stimulated by uterine palpation. Thus, it is not considered to be a viable way of improving pregnancy rate. It is expected that the application of drugs into recipients for the purpose of increasing pregnancy rates

Table 3. Comparison of pregnancy rate between FM and lidocaine treatments

Treatments		No. of recipients transfered	No.of pregnants at 35 days post-ET (%)
Heifers	FM	112	81 (72.3)[a]
	Lidocaine	108	73 (67.5)[b]
Cows	FM	98	48 (48.9)[a]
	Lidocaine	94	41 (43.6)[b]

[a,b] Values with different superscripts are different ($p<0.05$). FM-treated hifers were given 250 mg of flunixin meglumin (FM) i.m. at the time of ET; Lidocaine-treated heifers were given 5 ml lidocain; Pregnant diagnosis were carried out at Day 35 post-ET with ultrasonography.

after ET can instead decrease pregnancy rates due to the pain and stress associated with the injection and the following excitement. But for an experienced operator, who can transfer an embryo to the optimal site with minimal stimulation of the recipients, FM treatment may be a better option, as it inhibits $PGF_{2\alpha}$ production, than the use of a local anesthetic such as lidocaine.

The current study shows that an anti-luteolytic agent treatment such as FM rather than hCG treatment results in a higher pregnancy rate and natural recurrent estrus in infertile individuals in the ET. It is also considered desirable to provide a natural pregnancy environment by inhibiting the production of $PGF_{2\alpha}$, which negatively affects the growth and implantation of an embryo, rather than by artificially increasing P4 in normal individuals. Even when the lidocaine treatment was applied to stabilize recipients, the pregnancy rate was higher in FM-treated individuals, and it is also believed to be affected by anti-luteolysis. Consequently, an anti-luteolytic agent treatment such as an FM treatment resulted in a higher pregnancy rate, pregnancy maintenance and better estrous recurrence than hCG treatment, lidocaine treatment and no treatment whatsoever in the trials of ET.

However, it is clear that more studies with larger study groups and a greater repetition of the experiments will be needed to fully establish the advantages and disadvantages of luteotrophins including hCG administration on the pregnancy rate, pregnancy maintenance and reproductive cycle.

REFERENCES

Bogacki M, Silvia WJ, Rekawiecki R and Kotwica J. 2002. Direct inhibitory effect of progesterone on oxytocin-induced secretion of prostaglandin F_2alpha from bovine endometrial tissue. Biol. Reprod. 67:184-188.

Brackett BB and Oliphant G. 1975. Capacitation of rabbit spematozoa *in vitro*. Biol. Repod. 13:260-274.

Butler WR, Calaman JJ and Beam SW. 1996. Plasma and milk urea nitrogen in relation to pregnancy rate in lactating dairy cattle. J. Anim. Sci. 74:858-865.

Chagas e Silva J and Lopes da Costab L. 2005. Luteotrophic influence of early bovine embryos and the relationship between plasma progesterone concentrations and embryo survival. Theriogenology 64(1):49-60.

Chagas e Silva J, Lopes da Costa L and Robalo Silva J. 2002. Plasma progesterone profiles and factors affecting embryo-fetal mortality following embryo transfer in dairy cattle. Theriogenology 58:51-59.

Diaz T, Schmitt EJ, de la Sota RL, Thatcher MJ and Thatcher WW. 1998. Human chorionic gonadotropin-induced alterations in ovarian follicular dynamics during the estrous cycle of heifers. J. Anim. Sci. 76:1929-1936.

Dunne LD, Diskin MG, Boland MP, O'Farrell KJ and Sreenan JM. 1999. The effect of changes in feed intake around the time of insemination on systemic progesterone and embryo development and survival in cattle. In: M.G. Diskin, Editor, Fertility in the High-Producing Dairy Cow. vol. Ⅱ, no. 26, British Society of Animal Science Occasional Publication 381-384.

Ferguson JKW. 1941. A study of the motility of intact uterus at term. Surg. Gynecol. Obste. 73:359-366.

Garrett JE, Geisert RD, Zavy MT and Morgan GL. 1988. Evidence for maternal regulation of early conceptus growth and development in beef cattle. J. Reprod. Fertil. 84:437-446.

Hasler JF, Bowen RA, Nelson LD and Seidel GE. 1980. Serum progesterone concentrations in cows receiving embryo transfers. J. Reprod. Fertil. 58:71-77.

Helmer SD and Britt JH. 1986. Fertility of dairy cattle treated with human chorionic gonadotropin (hCG) to stimulate progesterone secretion. Theriogenology 5:683-695.

Kastelic JP, Bergfelt DR and Ginther OJ. 1991. Ultrasonic detection of the conceptus and characterization of intrauterine fluid on days 10 to 22 in heifers. Theriogenology 35:569-581.

Kenny DA, Boland MP, Diskin MG and Sreenan JM. 2001. Effect of pasture crude protein and fermentable energy supplementation on blood metabolite and progesterone concentrations and on embryo survival in heifers. Anim. Sci. 73:501-511.

Kerbler TL, Buhr MM, Jordan LT, Leslie KE and Walton JS. 1997. Relationship between maternal plasma progesterone concentration and interferon-tau synthesis by the conceptus in cattle. Theriogenology 47:703-714.

Lukaszewska J and Hansel W. 1980. Corpus luteum maintenance during early pregnancy in the cow. J. Reprod. Fertil. 59:485-493.

Mann GE and Lamming GE. 1995. Progesterone inhibition of the development of the luteolytic signal in cows. J. Reprod. Fertil. 104:1-5.

Mann GE, Lamming GE, Robinson RS and Wathes DC.

1999. The regulation of interferon-t production and uterine hormone receptors during early pregnancy. J. Reprod. Fertil. Suppl. 54:317-328.

Nishigai M, Kamomae H, Tanaka T and Kaneda Y. 2002. Improvement of pregnancy rate in Japanese Black cows by administration of hCG to recipients of transferred frozen-thawed embryos. Theriogenology 58:1597-1606.

Nishigai M, Kamomae H, Tanaka T, Kaneda Y. 1997. Effect of xylazine tranquilization during embryo transfer to bovine recipients on pregnancy rates. J. Reprod. Dev. 43: 41-46.

Nishigai M, Kamomae H, Tanaka T, Kaneda Y. 2001. The effect of human chorionic gonadotropin on the development and function of bovine corpus luteum. J. Reprod. Dev. 47:283-294.

Nishigai M. 2003. The development and prevalence of the transfer technique for frozen-thawed embryos of Japanese black beef cattle in tochigi prefecture. Journal of Reproduction and Development 49(1):23-36.

Price CA and Webb R. 1989. Ovarian response to hCG treatment during the oestrous cycle in heifers. J. Reprod. Fertil. 86:303-308.

Purcella SH, Beala WE, and Grayb KR. 2005. Effect of a CIDR insert and flunixin meglumine, administered at the time of embryo transfer, on pregnancy rate and resynchronization of estrus in beef cattle. Theriogenology 64(4): 867-878.

Santos JEP, Thatcher WW, Pool L and Overton MW. 2001. Effect of human chorionic gonadotropin on luteal function and reproductive performance of high-producing lactating Holstein dairy cows. J. Anim. Sci. 79:2881-2894.

Schmitt EJP, Barros CM, Fields PA, Fields MJ, Diaz T and Kluge JM *et al.*. 1996. A cellular and endocrine characterization of the original and induced corpus luteum after administration of a gonadotropin-releasing hormone agonist or human chorionic gonadotropin on day five of the estrous cycle. J. Anim. Sci. 74:1915-1929.

Schrick FN, Hockett ME, Towns TM, Saxton AM, Wert NE and Wehrman ME. 2001. Administration of a prostaglandin inhibitor immediately prior to embryo transfer improves pregnancy rates in cattle. Theriogenology 55:370.

Schrick FN, Scenna FN, Edwards JL, Hockett ME and Saxton AM. 2003. More evidence for a direct interaction between prostaglandin $F_{2\alpha}$ and development of bovine embryos. Proceedings of the CETA and AETA Joint Annu. Conv. Calgary, Alberta 43-52.

Seals RC, Lemaster JW, Hopkins FM and Schrick FN. 1998. Effects of elevated concentrations of prostaglandin $F_{2\alpha}$ on pregnancy rates in progestogen supplemented cattle. Prostaglandins 56:377-389.

Shemesh M, Milaguir F, Ayalon N and Hansel W. 1979. Steroidogenesis and prostaglandin synthesis by cultured bovine blastocysts. J. Reprod. Fertil. 56:181-185.

Sianangama PC and Rajamahendran R. 1992. Effect of human chorionic gonadotrophin administered at specific times following breeding on milk progesterone and pregnancy in cows. Theriogenology 38:85-96.

Smith AK, Broadbent PJ, Folman DF, Grimmer SP, Davies DAR and Dobson H, Norgestomet implants. 1996. plasma progesterone concentrations and embryo transfer pregnancy rates in cattle. Vet. Rec. 139:187-191.

Spell AR, Beal WE, Corah LR and Lamb GC. 2001. Evaluating recipient and embryo factors that affect pregnancy rates of embryo transfer in beef cattle. Theriogenology 56:287-297.

Sreenan JMBeehan D, Diskin MG. 1987. Factors affecting pregnancy rate following embryo transfer in the cow. Theriogenology 27:99-113.

Starbuck GR, Darwash AO, Mann GE and Lamming GE. 1999. The detection and treatment of post insemination progesterone insufficiency in dairy cows. In: M.G. Diskin, Editor, Fertility in the High-Producing Dairy Cow vol. II, no. 26, British Society of Animal Science Occasional Publication 447-450.

Stubbings RB and Walton JS. 1986. Relationship between plasma progesterone concentrations and pregnancy rates in cattle receiving either fresh or previously frozen embryos. Theriogenology 26:145-155.

Tervit HR, Cooper MW, Goold PG, Haszard GM. 1980. Non-surgical embryo transfer in cattle. Theriogenology 13: 63-71.

Thatcher WW, Guzeloglu A, Mattos R, Binelli M, Hansen TR and Pru JK. 2001. Uterine-conceptus interactions and reproductive failure in cattle. Theriogenology 56:1435-1450.

Walton JS, Halbert GW, Robinson NA and Leslie KE. 1990. Effects of progesterone and human chorionic gonadotropin administration five days post insemination on plasma and milk concentrations of progesterone and pregnancy rates of normal and repeat breeder dairy cows. Can. J.

Vet. Res. 54:305-308.

Wann RA and Randel RD. 1990. Effect of uterine manipulation 35 days after parturition on plasma concentrations of 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ in multiparous and primiparous Brahman cows. J. Anim. Sci. 68:1389-394.

Wathes DC, Robinson RS, Mann GE and Lamming GE. 1998. The establishment of early pregnancy in cows. Reprod. Domest. Anim. 33:279-284.

Wolf E, Arnold GJ, Bauersachs S, Beier HM, Blum H and Einspanier R *et al.* 2003. Embryo-maternal communication in bovine-strategies for deciphering a complex cross-talk. Reprod. Domest. Anim. 38:276-289.

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