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Short Communication

Superovulation and embryo recovery in crossbred cows treated with single dose of FSH dissolved in polyvinyl pyrrolidone

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ABSTRACT

The present study was undertaken to determine the superovulatory response and embryo recovery in crossbred cows treated with single dose of FSH dissolved in poly vinyl pyrrolidone (PVP). Nine crossbred cows were superovulated using single dose of FSH dissolved in 30% PVP and fourteen crossbred cows were superovulated using FSH multidose dissolved in saline. Donor cows were flushed non-surgically on day 7 after fixed time insemination with good quality semen, embryos recovered, examined, graded and cryopreserved until transfer. There was no significant difference between the two treatments of FSH in crossbred cows for number of corpus luteum, unovulated follicles, embryos recovered and transferable embryos. The rate of transferable embryos obtained were 85.0% and 86.9% in crossbred cows treated with single dose of FSH dissolved in PVP and multiple dose of FSH dissolved in saline respectively.

Key words : Embryo recovery, FSH single dose, polyvinyl pyrrolidone, superovulation

Superovulation is a key step in the embryo transfer technology in cattle and requires administration of a gonadotrophin preparation that mimics the effect of follicle stimulating hormone (FSH). The gonadotrophin must be available long enough for the follicle to grow and attain final maturation of the oocyte and ensure normal fertilization and embryo development. Porcine FSH has a relatively short half-life (Domoustier *et al.*, 1988), thus it needs to be given frequently to result in good ovulation rates. Generally, FSH is injected twice daily in declining doses over a period of 3 or 4 days (Gracia *et al.*, 1982) and the total amount of FSH in each treatment regimen is usually 26 to 50 mg. However, the use of two daily intramuscular injections is costly, a technical inconvenience, can result in errors in dose and injection time and also stress the treated cows. Smith *et al.* (1973) used 30 per cent Poly Vinyl Pyrrolidone (PVP) as diluent for FSH in an attempt to prolong the absorption of FSH, given in one or two injections to produce multiple births in cows. The present study was undertaken to determine the superovulatory response and embryo recovery in crossbred cows treated with single dose of FSH dissolved in PVP and to compare with multiple dose of FSH dissolved in saline. Twenty-three crossbred cows (aged 4-8 years) were examined per rectum for the presence of corpus luteum to induce oestrus using Prostaglandin $F_2\alpha$ (Iliren, Hoechst Roussel, Holland). The superovulatory treatment using FSH was started on day 0 after the induced oestrus. Of the different types of Poly Vinyl Pyrrolidone, we used PVP (Sigma Chemicals Co., USA) with molecular weight of 40,000. 30% w/v of PVP was prepared by dissolving 33.4 g of PVP in 100 ml of distilled water and sterilized by autoclaving (Yamamoto *et al.*, 1994). Just before use, FSH (Folltropin-V, Vetrepharm, Canada) 400 mg NIH was dissolved in 1 or 1.5 ml saline and mixed well with 10 ml of 30% PVP and injected to nine crossbred cows deep intramuscularly as described by Suzuki *et al.* (1994). Fourteen crossbred cows were given FSH 400 mg NIH dissolved in 20 ml of saline and given in multiple doses (twice daily for four days). All the cows were given Prostaglandin $F_2\alpha$ after 48 hours of the FSH single dose administration in PVP and multiple dose in saline. At 48 hours after the $PGF_2\alpha$ treatment all the donor cows were inseminated with frozen semen twice daily for two days at 12 hours interval (fixed time insemination) and superovulatory response were

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assessed by rectal palpation. Embryos were recovered on day 7 after the artificial insemination and the uterine horn was flushed non surgically by employing Foley balloon catheter with approximately one litre of "Bio Life Advantage medium" (Agtech Inc., USA). During flushing the embryos were recovered into an embryo filter or embryo recovery system. Embryos were examined under zoon stereo microscope and evaluated morphologically as transferable (late blastocyst and early embryo) and non-transferable (less or no germ cells inside the embryo) embryos as described by Shea (1981). The good quality embryos were loaded in 0.25 ml French straws and cryopreserved in liquid nitrogen until transfer. All the collected data were analyzed statistically by using student's `t' test (Snedecor and Cochran, 1989). The mean±SE number of corpus luteum, unovulated follicles, embryos recovered, transferable embryos were 4.54±1.33, 0.12±0.09, 2.23±1.33, 1.85±0.93 and 7.16±1.07, 0.23±0.21, 3.21±0.72, 2.82±0.72 in single dose of FSH dissolved in PVP and multiple dose of FSH dissolved in saline respectively (Table 1). There was no significant difference between the two FSH treatments in the number of corpus luteum, unovulated follicles, embryos recovered and transferable embryos in crossbred cows. The results obtained in this study concurred with earlier reports of Singh *et al.* (1998) and Murugavel *et al.* (1999) for crossbred cows. The rate of transferable embryos in crossbred cows treated with single dose FSH dissolved in PVP and multiple dose of FSH dissolved in saline were 85.0% and 86.9% respectively. In the present study, the rate of transferable embryos were higher than the previous reports of Yamamoto *et al.* (1994), Singh *et al.* (1998) and Murugavel *et al.* (1999). The superovulatory response measured by palpation of number of corpus luteum in the ovary and comparing to the actual number of ova or embryos recovered, there was variation and making palpation an unreliable method (Donaldson, 1985). Recovery of total and transferable embryos per donor in this study concurred with early reports of Murugavel *et al.* (199) for crossbred cows. In this study, there was low recovery of viable embryos when compared to the actual number of corpus luteum palpated which may be due to fertilizationfailure, embryo losses before uterine flushing and also post fertilization factors. The most important potential cause of variation in superovulatory response in cows to exogenous gonadotrophins may be inherent variability in ovarian follicular populations (Monneaux *et al.*, 1983). Smith *et al.* (1973) obtained a calving rate of 121% when they dissolved PVP with 5 mg FSH to produce multiple births in cows. The half life of FSH dissolved in cows. The half life of FSH dissolved in PVP was prolonged in the circulation of superovulated cows thus making the gonadotrophic functions more effective (Yamamoto *et al.*, 1994). The findings in this study indicated that single dose of FSH dissolved in PVP may be used effectively than the multidose FSH because of of stress condition to the cow was avoided. PVP is soluble in waer or in a wide range of organic solvents, which was different from other high molecular weight polymers. However, further studies are required to evaluate the function and use of poly vinyl pyrrolidone for superovulation in crossbred cows.

Table 1. Mean±SE superovulatory response and embryo recovery in crossbred cows

Treatment with FSH 400 mg NIH	Single dose dissolved in poly vinyl pyrrolidone (PVP)	Multiple dose dissolved in saline
Number of cows	9	14
Number of Corpus luteum	4.54±1.33[a]	7.16±1.07[a]
Number of unovulated follicles	0.12±0.09[a]	0.3±0.21[a]
Number of embryos recovered	2.23±1.33[a]	3.21±0.72[a]
Number of transferable embryos	1.85±0.93[a]	2.82±0.72[a]
Rate of transferable embryos	85.0 (17/20)	86.9 (40/46)
Rate of non-transferable embryos	15.0 (3/20)	13.1 (6/26)

a - do not differ significantly

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REFERENCES

Domoustier, M., Beckers, J.E., Zwalmen, V.D.P., Closset, J., Gillard, J.L. and Ectors, F. (1988). Determination of porcine plasma Folltropin levels during superovulation treatment in cows. Theriogenology, **30**: 379-386.

Donaldson, L.E. (1985). Estimation of superovulation responses in donor cows. *Vet. Record*, **117**: 33-38.

Gracia, G.J.K., Seidel, G.E. and Elsden, R.P. (1982). Efficacy of shortened FSH treatment for superovulating cattle. Theriogenology, 17: 90 (Abstr.).

Monneaux, D., Chupin, D. and Sanumande, J. (1983). Superovulatory responses of cattle. Theriogenology, 19: 55-61.

Murugavel, K., Ramalingam, S. and Ravindran, P. (1999). Superovulatory response and embryo recovery in crossbred cows under field condition. Indian J. Anim. Reprod., 20: 22-23.

Shea, B.F. (1981). Evaluating the bovine embryo. Theriogenology, 15: 41-42.

Singh, S.K., Agarwal, S.K. and Shankar, U. (1998). Effect of superovulatory response on embryo recovery and their quality in crossbred cattle. Indian J. Anim. Reprod., 19: 1-3.

Smith, L.E., Sitton, G.D. and Vincent, C.K. (1973). Limited injections of follicle stimulating hormone for multiple births in beef cattle. J. Anim. Sci., 37: 423-527.

Snedecor, G.W. and Cochran, W.G. (1989). *Statistical Methods*. 8th edn., Iowa State University Press, Ames, Iowa.

Suzuki, T., Yamamoto, M. and Takagi, M. (1994). Superovulation of beef cows and heifers with a single injection of FSH diluted in PVP. Vet. Rec., 135: 41-42.

Yamamoto, M., Ooe, M., Kawaguchi, M. and Suzuki, T. (1994). Superovulation in the cow with a single intramuscular injection of FSH dissolved in polyvinyl pyrrolidone. Theriogenology, 41: 747-755.