SUPEROVULATORY RESPONSE IN CROSSBRED COWS WITH MULTIPLE VERSUS SINGLE INJECTION OF FSH DISSOLVED IN POLYVINYLPYRROLIDONE

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ABSTRACT

The use of a single FSH injection for inducing superovulation in cow is desirable. In this study we examined whether dissolving FSH in polyvinylpyrrolidone (PVP) would reduce the rate of absorption of FSH and allow it to be administered in a single dose for superovulation. Crossbred cows (n= 9 in each group) were superovulated either with a single intra-muscular injection of 18 mg FSH dissolved in 10 ml of 30 % PVP or with 18 mg of FSH dissolved in saline given as eight intra-muscular injections over four days period. Prostaglandin $F_{2\alpha}$ was given to all the cows 48 h after the first FSH injection. The cows were inseminated with frozen semen at 12 and 24 h following the estrus onset. Superovulatory response was assessed by palpation per rectum on day 7 post breeding and the cows with more than 2 corpus luteum (CL) were flushed. Progesterone level in the blood samples collected on the day of first FSH injection and on the day of embryo collection were found to be significantly ($P<0.05$) higher in multiple injection group (7.3 ± 1.4 and 22.6 ± 4.6 ng/ml) compared to the single injection group (2.77 ± 1.15 and 10.9 ± 3.3 ng/ml). Nevertheless the number of transferable embryos in multiple injection group (1.9 ± 0.8) and single injection group (1.0 ± 0.8) did not differ significantly. The study revealed that 18 mg of FSH was not sufficient to induce satisfactory superovulation in crossbred cows through the single PVP-FSH injection protocol or conventional multiple FSH injections protocol.

Keywords : Superovulation, Cows, FSH, PVP.

INTRODUCTION

Superovulation is a key element for embryo transfer programme in cattle. Superovulation in cattle requires exogenous administration of a gonadotropin preparation, which is rich in or mimicking the effect of FSH. During superovulation, gonadotropin should be available for a long time for continuous follicle growth and final maturation of oocyte, which will ensure normal fertilization and embryo development (Yamamoto *et al.*, 1994). Pregnant mare serum gonadotropin (PMSG) or FSH is commonly used for superovulatory treatment in cattle (Boland *et al.*, 1991; Goulding *et al.*, 1991; Datta *et al.*, 1992; Agarwal *et al.*, 1993). Due to longer half-life, a single injection of PMSG has been found to be sufficient for inducing superovulation in cattle (Nagajima *et al.*, 1992). Nevertheless, the continuous FSH like activity of PMSG even after the induced estrus could adversely affect the uterine environment by increasing estrogen level through the stimulation of follicles, which ultimately detoriates embryo quality (Nagajima *et al.*, 1992). In contrast, the half-life of FSH is relatively shorter and 2 injections /d over a period of 4 d are required to induce superovulation in cattle (Demoustier *et al.*, 1988).

It is technically inconvenient to inject multiple FSH doses over a long period as it may results in erroneous dose and time of injection. Besides, the reconstituted

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FSH preparation is needed to be stored at refrigeration temperature until the completion of the treatments, which causes difficulties for carrying out superovulation at field level. Moreover, frequent treatments and excessive handling may induce stress in donor animals and reduce superovulatory response (Edwards *et al.*, 1987). An efficient and simple superovulation protocol is expected to reduce the donor-handling costs and improve superovulatory response. Several attempts have been made previously to prolong the absorption of a single FSH injection. However, it is evident that the use of 1% carboxymethyl cellulose (Mills *et al.*, 1971) and gelatine-saline vehicle (Looney *et al.*, 1981) to prolong the FSH absorption does not produce encouraging results.

Polyvinylpyrrolidone (PVP) is a high molecular weight polymer. PVP is capable to form complex with a substance when added to it and can prolong the functional time of that substance *in vivo* (Yamamoto *et al.*, 1994). It has been proposed that PVP can be used as a vehicle for FSH to induce superovulation in cattle (Taya and Sasamoto 1989, Suzuki *et al.*, 1994 and Yamamoto *et al.*, 1994). The aim of the present study was to investigate the suitability of PVP as a vehicle for FSH to induce superovulation in crossbred cows using a single injection protocol. The hypothesis was that the number and quality of embryos recovered using a single PVP-FSH injection would be comparable with that recovered using multiple FSH injections.

MATERIALS AND METHODS

Experimental animals and groups

Eighteen healthy and cyclic multiparous crossbred cows (Sindhi × Jersey) were selected from the herd of Livestock Research Station of Tamil Nadu Veterinary and Animal Sciences University, India. The animals were randomly allocated into 2 groups (9 in each), FSH multiple injection (FSH-MI) group and FSH single injection (FSH-SI) group.

Estrus synchronization and superovulation

The PVP 40 with molecular weight 40,000 (Sigma-Aldrich Co. St. Louis, MO, USA) was used for the study. PVP solution was made by dissolving 33.4 g of PVP in 100 ml double distilled water, autoclaved and stored at 4°C in aliquots of 10 ml. The fresh FSH-PVP solution was made before each treatment. Briefly, 18 mg of FSH (Folltropin-V; Vetripharm, Inc., ON, Canada; equivalent to 400 mg NIH-FSH-p) was dissolved in 1.5 ml normal saline and mixed well with 10 mL PVP solution. Estrous cycle of the experimental animals was synchronized with double intra-muscular injections of 25 mg of Prostaglandin $F_{2\alpha}$ (PGF2á, Lutalyse, Upjohn, Belgium) at 11 d interval. Superovulatory treatment was initiated on day 10 of the synchronized cycle. All the cows in FSH-MI group received 2 intra-muscular injections of FSH daily at 12 h interval over a period of 4 d at decreasing dose rate (3.6, 2.7, 1.8 and 0.9 mg respectively). Luteolysis was induced by injecting 25 mg of $PGF_{2\alpha}$ at 48 h following the first FSH injection. All the cows in FSH-SI group received a single intra-muscular injection of FSH-PVP solution (containing 18 mg of FSH) and the luteolysis procedure was similar to that described for FSH-MI group. Estrus detection in experimental animals was done by bull parading and it was confirmed by palpation per rectum. The animals were inseminated with frozen semen at 12 and 24 h following the estrus onset.

Embryo collection and plasma progesterone determination

Embryo flushing was done non-surgically on day 7 (day 0 was the day of superovulatory estrus) of the estrous cycle using a two-way foley catheter with an inflatable balloon. The cows with more than 2 corpus luteum (CL) were flushed. The catheter was inserted into the uterine body and the balloon was inflated just cranial to cervix. Modified Dulbecco's phosphate buffered saline (DPBS; supplemented with 0.1% BSA) was used as flushing medium. Approximately 40 to 60 ml of the flushing medium was allowed to flow into uterus and the inlet was closed. Simultaneously the outlet tube was opened and the flushing media was passed through an embryo filter (70 µm pore size; Emcon-Immuno Systems, Spring Valley, WI, USA). The flushing was done repeatedly and approximately 500 ml of flushing media was used for each animal. The content of the embryo filter was washed thoroughly with DPBS (supplemented with 0.1% BSA) and transferred to a

disposable petridish (90 mm diameter) with grid and screened thoroughly for the presence of embryos under a Nikon zoom-stereo microscope (SMZ800). Embryos were picked up using a 10 µl unopettes (Becton Dickinson, Rutherford, NJ, USA) and transferred to a disposable petridish (35 mm diameter) containing holding media (DPBS supplemented with 4% fetal calf serum). The quality of an embryo (excellent, good, fair and poor) was determined on the basis of morphological characters (Shea 1981; Linder and Wright 1983). The excellent and good quality embryos were considered as transferable embryos and fair and poor quality embryos were considered as non-transferable embryos (Kathiresan *et al.*, 1994). For determining plasma progesterone concentration, blood samples were collected on the day of first FSH injection, PGF2á injection, superovulatory estrus and embryo collection. Plasma progesterone level was determined using a solid phase radio-immuno assay kit (PROG-CTK-4, DiaSorin, Saluggia, Italy) according to a previously described method (Arosh *et al.*, 2000). The mean intra- and inter-assays CVs were less than 10%.

Statistical analysis

The data were analyzed using the SPSS 10.0.1 software package (SPSS Inc., Chicago, Illinois, USA) and presented as mean ± S.E.M. The data on interval between the $PGF_{2\alpha}$ injection and estrus onset, estrus duration, number of CL, anovulated follicles, transferable embryos, non-transferable embryos and unfertilized oocytes were analyzed by Student's t-test. The variation in plasma progesterone level was analyzed using ANOVA followed by multiple pairwise mean comparisons using Student-Newman-Keuls test. The model included group, stages of superovulation and interaction between group and stage as sources of variation.

RESULTS AND DISCUSSION

The superovulatory estrus was detected in 100% (9 out of 9) experimental animals in FSH-MI group and 66.6% (6 out of 9) experimental animals in FSH-SI group. The interval (h) between the $PGF_{2\alpha}$ injection and estrus onset was found to be 52.0 ± 2.0 and 54.7 ± 2.1, respectively in FSH-MI and FSH-SI group, while the duration of estrus (h) was found to be 32.0 ± 2.0 and 29.3 ± 2.1, respectively. The onset and duration of estrus did not differ significantly between the groups.

Total number of CL was found to be significantly ($P<0.05$) higher in FSH-MI group, but the number of anovulated follicles did not differ significantly between the groups (Table). It was observed that in FSH-MI group all the cows had more than 2 CL, but in FSH-SI group only 3 cows had more than 2 CL. The number of transferable and non-transferable embryos and unfertilized oocytes did not differ significantly between the groups (Table). In FSH-MI group, the rates of excellent, good, fair and poor quality embryos were 12.5, 58.3, 0.0 and 29.2% respectively. The corresponding values in FSH-SI group were 77.8, 22.2, 0.0 and 0.0%.

The effect of group, stages of superovulation and interaction between group and stage was found to be significant ($P<0.05$). In both the groups, plasma progesterone level was found to be lowest ($P<0.05$) on the day of superovulatory estrus and highest ($P<0.05$) on the day of embryo collection. Progesterone level was found to be much higher in FSH-MI group (22.6 ± 4.6 ng/ml) compared to FSH-SI group (10.9 ± 3.3 ng/ml) on the day of embryo collection. In FSH-SI group, progesterone level was found to be less than 1 ng/ml in 3 animals on the day of embryo collection.

The development of an efficient and simple superovulation protocol is desirable to reduce the donor-handling costs and improve the recovery of good quality embryos. Though a single injection of PMSG and multiple FSH injections are commonly used for superovulation in cattle, but these techniques have some limitations. In this study, we assessed the efficiency of a single PVP-FSH injection for superovulation in crossbred cows. The results indicated that a single PVP-FSH injection containing 18 mg of FSH was not sufficient to induce satisfactory superovulation in crossbred cows.

The solubility in water and wide range of organic solvents is a unique property of PVP compared to the other high molecular weight polymers. Moreover, PVP is capable to form complex with several compounds such as pigments, iodide, antibiotics and insulin (FAO

1966). Therefore, PVP may be used as a vehicle for many biological compounds to prolong their functional time *in vivo* (FAO, 1966). It is proposed that PVP may be used as a vehicle for FSH to induce superovulation in cattle (Suzuki *et al.*, 1994). In this study, we examined if the half-life of FSH could be extended in the circulation of superovulated crossbred cows by dissolving it in PVP solution.

The average number of CL was found to be significantly higher in animals treated with multiple FSH injections compared to the single PVP-FSH injection. Simultaneously, in multiple FSH injection group, 100% (9 out of 9) animals had more than 2 CL and in single PVP-FSH injection group only 33% (3 out of 9) animals had more than 2 CL. Though the number of transferable and non-transferable embryos did not vary between the treatment groups, the values were found to be comparatively higher in animals treated with multiple FSH injections. The results indicated that the superovulatory response was better with multiple FSH injections compared to single PVP-FSH injection. In contrast, it is reported that a single injection of 30 mg FSH dissolved in PVP improves the yield of transferable embryos compared to the conventional multiple FSH injections (Yamamoto *et al.*, 1994; Takedomi *et al.*, 1995).

It was observed that the yield of transferable embryos was much lower in both the groups compared to a previous report using single dose of 30 mg FSH-R in 30% PVP and 28 mg FSH-R in multiple divided doses (Yamamoto *et al.*, 1994). The poor yield of good quality embryos in both the groups might be due the fact that the FSH dose that used in the experiments was not sufficient to induce satisfactory superovulation in crossbred cows (Yamamoto *et al.*, 1995). Plasma progesterone level was found to be significantly higher on the day of $PGF_{2\alpha}$ injection compared to the day of first FSH injection. The increased progesterone level

Table: Superovulatory response in crossbred cows following the multiple injections (FSH-MI) and single injection (FSH-SI)

Parameters	FSH-MI		FSH-SI	
	Range	Mean ± S.E.M.	Range	Mean ± S.E.M.
Number of corpus luteum	2 - 13	7.3 ± 1.4 a	0 - 8	2.77± 1.15 b
Number of anovulated follciles	0 - 1	0.1 ± 0.1	0 - 3	0.8 ± 0.4
Number of transferable embryos	0 - 6	1.9 ± 0.8	0 - 7	1.0 ± 0.8
Number of non-transferable embryos	0 - 7	0.8 ± 0.8	0	0
Number of unfertilized oocytes	0 - 6	0.7 ± 0.7	0	0

Values with different superscript within row differ significantly ($P<0.05$).

following the FSH injection might be due to the luteotropic effect of FSH (Lindsell *et al.*, 1986). The significantly higher progesterone level on the day of embryo collection in the multiple FSH injection group might be due to the presence of more number of CL. In the single PVP-FSH injection group, 3 animals did not express superovulatory estrus on the expected day and had high plasma progesterone level (more than 1.28 ng/ml). These animals expressed estrus and had anovulated follicles and low plasma progesterone level (less than 1 ng/ml) on the expected day of embryo collection. The results indicated that these animals probably did not respond to the $PGF_{2\alpha}$ induced luteolysis during superovulation.

In conclusion, 18 mg of FSH was not sufficient to induce satisfactory superovulation in crossbred cows either through the single PVP-FSH injection protocol or conventional multiple FSH injections protocol. We suggest further studies using the different doses of PVP-FSH to prolong the half life of FSH for efficient superovulation in crossbred cows.

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