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Dose-related Effects of Follicle Stimulating Hormone on Superovulation in Indigenous Cows of Bangladesh

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SUMMARY

The present study aimed at determining the effective dose of Folltropin, a follicle timulating hormone (FSH), on superovulation in indigenous cows of Bangladesh. Fifteen regularly cycling 5~7 years old dry cows, weighing 200~250 kg with 2.5~3.0 body condition scores (BCS) were divided into three groups (n = 5). Individual groups were superovulated with 100, 200 or 300 mg of Folltropin per animal. The superovulation treatment was initiated at Day 10 or Day 11 of the estrous cycle (Day 0 = day of estrus). Alfaprostol (6 mg) was injected to each cow 72 h after the initiation of superovulation treatment to induce eestrus. After confirming standing estrus, the cows were inseminated 2~3 times, 12 h apart, depending on the duration of estrus. At Day 6 or Day 7, individual horns of the uterus were flushed with 150~200 ml of phosphate buffered saline supplemented with BSA (0.2%), penicillin (100 IU/ ml) and streptomycin (100 μg/ ml) using a two-way foley catheter. The embryos were concentrated, removing the excess medium through an embryo filter, and identified under a stereomicroscope. The identified embryos were collected, washed four times, evaluated and graded as excellent, good, fair or poor. The excellent, good and fair embryos were considered as transferable quality embryos.

The mean (range) numbers of embryos collected vs. transferable quality embryos for 100, 200 and 300 mg of Folltropin were 4.5 (1~10) vs. 3.5 (1~8); 2.5 (1~4) vs. 1 (0~2) and 0.0 (0~0) vs. 0.0 (0~0), respectively. Folltropin at a dose of 100 or 200 mg produced suitable ovarian stimulation for superovulation in indigenous zebu cows of Bangladesh. A dose of 300 mg or more Folltropin consistently caused preovulatory corpora lutea formation in the ovaries and resulted in zero embryo recovery.

(Key words : FSH, superovulation, embryos, zebu cows)

INTRODUCTION

Multiple ovulation and embryo transfer technology has been introduced in to the indigenous zebu cows of Bangladesh since 1998. Feeding regime and optimum body condition score of the donor animals was standardize in our laboratory and the result showed that donor animals with body condition score (BCS) 2.5~3.0 are suitable for embryo

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production (Siddiqui et al., 2002). Possible dose effect of pregnant mare serum gonadotropin (PMSG) has also been studied and the result indicated that 1500 to 2000 IU of PMSG produced best superovulatory response in indigenous zebu cows of Bangladesh (Khan et al., 2000).

Follicle stimulating hormone is a popular gonadotrophin preparation for superovulating the cows due to its consistent production of high quality transferable embryos (Elsden et al., 1978; FAO, 1991). The Folltropin is a widely used brand of FSH with satisfactory embryo yield (Tribulo et al., 1993; Walsh et al., 1993). The dose of Folltropoin for superovualtion is 400 mg for *Bos taurus* cattle (Hasler et al., 1983; Fernandez et al., 1993). A dose of 800 mg often interrupted luteal regression as demonstrated by high blood progesterone levels at estrus. Improper dose of superovulatory drug may result in abnormalities of the follicular development and oocyte maturation, abnormalities of ovulation like premature ovulation, unovulated follicles, unovulated luteinized follicles and premature luteal regression (Kafi and McGowan, 1997). The doses of Folltropin need to be standardized for individual breeds because the breeds of cows influence the superovulatory response (Breuel et al., 1991). The zebu cows in Bangladesh are small in size with 200~300 kg body weight. The doses of Folltropin used for *Bos taurus* may not be suitable for such small zebu cattle. Therefore, the present study was conducted with the aim of determining the effective dose of Folltropin for the best superovulatory responses in Bangladeshi zebu cows.

MATERIALS AND METHODS

Fifteen regularly cycling nondescript indigenous zebu cows of 5 to 7 years old, weighing 200~250 kg, 1 to 3 parity and 2.5 to 3.0 body condition score (1 to 5 scales) were included in the present study. The cyclicity of the cow was monitored following the behavioral and clinical signs of oestrus and by assessing the ovarian function per-rectum. There were no developmental disorders or detectable genital diseases or unusual peri-parturent events those could have influenced the superovulatory response in these selected cows.

The cows were housed in a well-ventilated, concrete floor, tin-roofed shed. A combination of roughage and concentrate feed was supplied to the cows measuring 9 kg roughage and 1.5 kg concentrate and amounting a total of about 39.6 MJ metabolizable energy. Water supply was *ad libitum*.

The 100, 200 and 300 mg of Folltropin (Follitropin® Vetrepharm Canada Inc., London, Ontario, Canada) were used for individual animals in each of 3 groups (n = 5) of cows. The superovulation treatment was initiated at Day 10 or Day 11 of the oestrous cycle (Day 0 = day of oestrus). The total doses were divided into eight equal fractions. Individual dose fractions were injected intramuscularly on 12 hours interval, 0700h and 1900h. Each cow received an intramuscular injection of 10 ml vitamin AD_3E (Pantex Holland B.V., ZG Duizel, Holland) 2 to 3 weeks before initiation of superovulation treatment. A luteolytic dose of alfaprosteol (3 ml Gabbrostim®, VETEM S.p.A, Porto Empedocle, Italy) was injected to each donor animal 72 hours after initiation of superovulation i.e. with sixth fraction of Follitropin injection to induce oestrus.

After prostaglandin injection, the cows were observed for the onset of behavioral and clinical signs of oestrus. The cows were inseminated with frozen semen 2 or 3 times, starting from 6 hours after observed standing oestrus and at 12 hours apart, depending on the duration of oestrus.

Phosphate buffered saline with calcium and magnesium salts (PBS, Biochrom KG, Berlin, Germany) was supplemented with 0.2% bovine serum albumin (Albumin Concentrate®, 20% w/v, ICP, Aukland, Newzealand), penicillin (100 IU/ml)

and streptomycin (100 μg/ml) and used as flushing medium.

At Day 6 or Day 7 (day of first AI = day 1), both horns of the uterus were flushed using epidural anesthesia and sedation (Diazepam, 20 mg; SQUARE Pharmaceuticals Ltd, Dhaka, Bangladesh). The number of corpora lutea (CL) was estimated per rectum prior to flushing. A 2-way Foley catheter with Luer-Lok fittings ("Modell Neustadt/Aisch" type Rusch catheter, Minitub GmbH, Germany) was used for flushing the content of uterine horns. About 30~40 ml of medium was filled in to the uterus and aspirated back slowly using a 50 ml disposable syringe. A total of 150~200 ml of medium was used in five to six flushes of individual horns.

The embryos in flushing medium were concentrated by removing the excess medium through an embryo filter (22 μm pore size, EMCON®, Minitub GmbH, Germany) and were identified under a stereomicroscope (20X, Binocular Stereomicroscope ®, XTS-110, Ningbo Optical and Electrical Instrument Co. Ltd. Ningbo, China). The embryos were washed four times and evaluated subsequently.

The embryos were classified in four groups viz. excellent, good, fair and poor on the basis of morphological character (Linder and Wright, 1983). The excellent, good and fair embryos were considered as transferable quality embryos.

The data on number of corpora lutea palpated per cow, embryo recovery rate and the transferable embryos for different doses of FSH were compared by using t-test (Anon, 1996). The embryo recovery rate and the transferable embryos recovered were expressed in percentage.

RESULTS

The data on superovulatory responses of indigenous zebu cows after treatment with different doses of Folltropin are shown in the Table 1.

The treatment of cows with 300 mg of FSH had more (12.0) palpable corpora lutea at rectal palpation than that resulted after treatment with 200 mg (8.5) and 100 mg FSH (8.3); however, the differences were not significant (P>0.05).

Folltropin at a dose of 100 and 200 mg resulted in more embryos recovered (4.5 and 2.5, respectively) than it did at a dose of 300 mg (0.0). The use of 300 mg Folltropin resulted in zero embryo recovery. The number of embryos recovered after treatment with 100 mg and 200 mg FSH did not differ between each other (P>0.05). Moreover, only the cows that received 100 and 200 mg Folltropin yielded transferable embryos (3.5 and 1.0, respectively). Again, the difference in the transferable quality embryos between doses of 100 mg and 200 mg of Folltropin was not significant (P>0.05).

DISCUSSION

The results of the present experiment characterized ovarian response, embryo yield and embryo quality in indigenous zebu cows of Bangladesh at

Table 1. Superovulation and embryo production in response to different doses of Folltropin

Doses (mg)	Number of CL palpated mean (range)	Number of embryos recovered mean (range)	Number of transferable embryos mean (range)
100	8.3 (1~14)	4.5 (1~10)	3.5 (1~8)
200	8.5 (7~12)	2.5 (1~ 4)	1 (0~2)
300	12 (8~16)	0 (0~ 0)	0 (0~0; 00)

The difference between values of different doses was not significant (P>0.05).

superovulation with Folltropin. These results emphasize the significance of a proper dose of Folltropin for achieving improved ovarian response in zebu cows. The main findings of the present study is that a dose of 100~200 mg Folltropin is suitable for the superovulation treatment of local zebu cows in Bangladesh. The superovulatory responses were judged based on the number of corpora lutea palpated, number of embryos recovered and the number of transferable quality embryos.

Follicle stimulating hormone is a glycoprotein with low molecular weight and short half-life (2 to 2.5 h), necessitating repeated administration to cause superovulation (Walsh et al., 1993; Mahmoudzadeh, 1994). Eight equal doses of Foltropin over 4 days were used to induce superovulation. The Folltropin is a widely used brand of FSH with satisfactory embryo yield (Tribulo et al., 1993; Walsh et al., 1993). Tribulo et al., (1993) reported good superovulation and embryo recovery after treatment with 400 mg of Folltropin in Brahman -cross heifer. The results of the present study indicate that Folltropin at a dose of 300 mg resulted zero embryo recovery although the ovaries had good number of palpable corpora lutea. A dose of more than 200 mg induced high number of preovulatory CL formation. This may be due to smaller body size of Bangladeshi zebu cows compared with many other breeds of cattle.

The result of the present study indicated no relationship between the palpable number of CL and the number of ovulation. The occurrence of unovulated luteinized follicles is one of the common abnormalities that may compromise the superovulatory response in the bovine (Kafi and McGowan, 1997). Once the optimum dose is crossed, higher doses of gonadotrophin may result in ovarian overstimulation (Pawlyshyn et al., 1986; Wang et al., 1988). Unovulated luteinized follicles may result as a consequence of overstmulation of ovary (McGowan et al., 1985). Monniaux et al., (1983) found such luteal structure on the ovaries at day 7 or 8 after oestrus. Lueitinized follicles are often confusing on gross observation with corpora lutea. Monniaux et al., (1983) described the histology of these structures in the ovaries of a group of superovulated cattle slaughtered 48 h after the preovulatory LH surge. They found corpora haemorrhagica with partially ejected follicular fluid and the oocyte in the antrum. These follicles may become luteinized and be indistinguishable grossly from normal CLs at the time of embryo recovery.

Commercial FSH preparations are usually contaminated with variable amount of luteinizing hormone (LH). Excessive LH during the later stage of follicular growth is probably detrimental for normal maturation and ovulation of oocyte. Kafi and McGowan (1997) stated that resumption of meiosis in the oocyte at an inapproprite time, premature luteinization of follicles and down-regulation of LH receptors on theca and/or granulosa cells are responsible for the poor quality of embryos recovered from animals treated with Gonadotrophin with high level of LH.

The superovulatory effect of pituitary extract on the bovine ovaries depends on the ratio of follicle stimulating hormone to luteinizing hormone present in it (Chupin et al., 1984; 1985). Perhaps, the 300 mg of Folltropin used in this study was large for smaller zebu cows of Bangladesh. The excess amount of LH preparation might have caused luteinization of premature follicle, desensitization of LH receptor, premature ovulation and drastically decreased the superovulatory response as reported earlier (Boland et al., 1991; Breuel et al., 1991; Herrler et al., 1991). Over doses of FSH also result in the production of poor quality embryo (Mapletoft, 1986).

The main problem of superovulation in large monotocous species like cattle is the variability of their response to exogenous gonadotrophin treatment (Mahmoudzadeh, 1994). Hormones used for

superovulation, individual animals and environment are the factors responsible for this drawback. The performance of the donor cows is a major factor influencing the success of the overall procedure. Age, production levels, sanitary and reproductive history of the donor cows are, among others, important factors to be considered during interpretation of results from superovulation treatment (Hasler et al., 1983).

In conclusion, a dose of 100 or 200 mg of Folltropin is suitable for effective superovulatory response in indigenous zebu cows of Bangladesh and the administration of 300 mg of Folltropin consistently induced formation of multiple corpora lutea with zero embryo recovery.

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