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Superovulatory Response of Red Sindhi Cows (*Bos Taurus Indicus*) Treated with Three Different Doses of FSH

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Abstract: This study evaluated the superovulatory response of Red Sindhi cows to three different doses of FSH. Cows were randomly assigned to three experimental groups in which they received 100, 133 or 200 mg Folltropin-V® as superovulatory treatment, totaling 16, 13 and 14 superovulation per group, respectively. Superovulatory response was determined by the number of corpora lutea present in both ovaries on (D6) after first insemination and the number of recovered structures, percentage of recovered structures (recovered structures/CL), viable and freezable embryos collected on D7. The development stage of recovered structures was also examined. An effect of dose on the number of corpora lutea and number of recovered structures ($p<0.05$) was observed and the 200 mg dose produced the best results (6.4±0.85 and 3.5±0.95, respectively). Considering the number of viable embryos and the number of freezable embryos, there was no difference ($p>0.05$) among the three groups. Statistical difference was found between the stages of embryonic development with a greater number of embryos in the stage between compact morulae and blastocyst ($p<0.05$). Under the conditions of this study the dosage of 200 mg FSH induced higher number of corpora lutea and total recovered structures but the doses of FSH tested did not influence the number of viable and freezable embryos.

Key words: Bovine, embryos, superovulation, FSH, superovulatory, freezable embryos

INTRODUCTION

The breed Red Sindhi (*Bos taurus indicus*) is native to the Pakistani province of Sindh. Red Sindhi main features are the reddish coat color and dark mucous membranes, ideal for tropical and sub-tropical climates. The animals are small, have a lower absolute food consumption, good reproductive efficiency and good milk production yields in both quantity and quality. Besides, these advantages stand out due to the breed's excellent adaptability to adverse conditions of climate and management, especially concerning feeding. Because of its resistance to heat Red Sindhi is now distributed across many parts of India and at least in 33 countries in Asia, Africa, Oceania and the Americas (Talpur *et al*., 2006).

In Brazil, the breed adapted well to the semi-arid northeastern region where it is used in small farms in milk and meat production being productive even in adverse conditions (EMEPA, 2009) (Empresa Estadual de Pesquisa Agropecuaria da Paraiba S. A. Disponivel em: http://www.emepa.org.br/gado_sindi.php. Acess in August, 2009). Based on these attributes and performance of these animals, the wide use of Red Sindhi as a pure breed and in crossings with *Bos taurus taurus* breeds gains greater importance, since it results in productive, durable, small-sized livestock, recommended, specially for small farms typical of family agriculture.

Research involving the reproductive characteristics are scarce in this breed. Such studies are important to generate relevant information to reproduction technologies, to optimize and to accelerate the diffusion of the genetics of these animals. Among these techniques is embryo transfer which consists of the use of exogenous hormones to induce the growth of multiple follicles, thus, creating multiple oocytes that can be fertilized (Bulbul *et al*., 2013). This technique affords to achieve a higher number of embryos and therefore physiologically possible pregnancies, thereby increasing the number of offspring of females with more productive characteristics (Kugonza *et al*., 2013).

Currently, the results obtained in embryo transfer programs have varied, depending on the genetic group and breed used. Hasler (2003) reports an average of between 5.0 and 5.5 viable embryos per collection per donor when it comes to *Bos taurus taurus*. The genetic

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group *Bos taurus* indicus a greater variability occurs between breeds. As for Nellore the results vary between 8 and 9 viable embryos per collection (Baruselli *et al*., 2006; Nogueira *et al*., 2007) while in Gyr cows, the result is <3 and 5 viable embryos per collection (Prado *et al*., 2007).

Besides breed and genetic group, many other factors can influence the variability in the response to superovulatory treatment (Mapletoft *et al*., 2002, Vieira 2013). According to Kafi and McGowan (1997), this variation may be influenced by intrinsic factors such as age, breed and genetic group and presence of the dominant follicle or extrinsic factors such as environment, nutrition, sub-clinical infections and lactation.

The use of FSH as an inducer of superovulation has been extensively studied, like the different administration pathways (Bo *et al*., 1994; Kelly *et al*., 1997; Kanitz *et al*., 2002), the efficiency of commercial products of different brands and origins (Kelly *et al*., 1997; Costa *et al*., 2001), variations in the FSH: LH ratio between commercial preparations (Kanitz *et al*., 2002) and the use of different doses (Nilchuen *et al*., 2012).

Baruselli *et al*. (2006) tested doses of 100, 133 and 200 mg for superovulation of Nellore cows and found no difference in the number of viable embryos, showing that it is possible to reduce the dose and still get satisfactory results. On the other hand, Prado *et al*. (2007) superovulated Gyr cows with three FSH doses (300-500 IU) and reported differences in production of viable embryos. The intermediate dose showed better results than the other doses in Nellore.

MATERIALS AND METHODS

Location of the experiment and animals: The experiment was conducted at the Animal Reproduction Department of Animal Reproduction and Evaluation, Institute of Animal Science, Federal Rural University of Rio de Janeiro, located in Seropedica, Rio de Janeiro, Brazil (latitude: 22°44'S; longitude: 43°42'O; altitude: 26 m). The 16 not-lactating Red Sindhi cows aged between 4 and 10 years were used with body condition score between 3.5 and 4.0 (scale of 1-5) fed on pasture and supplemented with mineral salt ad libitum. The animals were homogeneously divided according to age between treatments, avoiding a possible effect of age on the results.

Experimental design: In order to determine the optimal FSH dose, cows were divided in three experimental groups in which they received 100, 133 and 200 mg Folltropin-V® (Bioniche Animal Helth, Ontario, Canada, where 1 mg corresponds to 1 mg of NIH-FSH-P1) as superovulatory treatment. Four replications were conducted in each experimental group, totaling 16, 13 and 14 superovulations in groups of 100, 133 and 200 mg Folltropin-V®, respectively. The superovulatory response was determined by the number of Corpora Lutea (CL) present in both ovaries on D6 after the AI and the number of recovered structures, percentage of recovered structures (structures recovered/CL), viable embryos and freezable embryos collected on D7 after AI. The stage of embryonic development of structures recovered were also evaluated.

Superovulation protocols: For the superovulation and fixed timed insemination of donors, the protocol P-36, described by Barros and Nogueira (2001), was utilized. The only change concerned the FSH dose, used according to treatment (100, 133 or 200 mg Folltropin-V®).

Treatment was initiated on a random D0 of the estrous cycle with the placement of an intravaginal device containing 1.9 g progesterone (CIDR-B®, Pfizer Animal Health, Sao Paulo, Brazil) and the intramuscular application of 2 mg estradiol benzoate (Estrogin®, Farmavet, Sao Paulo, Brazil) (D0). In D4 the administration of FSH in eight decreasing doses began, for 4 consecutive days at 12 h intervals. Concurrent with the 5th FSH dose the PGF2α analogue (0.5 mg of cloprostenol; Sincrosin®, Vallee, Montes Claros-MG, Brazil) was administered. The progesterone device was removed in the afternoon of D7 after artificial insemination. The 12 h after the last FSH injection 25 μg of GnRH (Gestran Plus®, ARSA SRL, Buenos Aires, Argentina) was administered. The artificial inseminations were performed 12 and 24 h after application of GnRH with cryopreserved semen of Red Sindhi bulls (Fig. 1). About 6 days after the 1st artificial insemination an ultrasound examination (Philips-VMI-510-V2, 7.5 MHz) of the ovaries was carried out to determine the number of CL.

Collection and evaluation of embryos: Embryo recovery was performed by uterine flushing non-surgical method on D7. All procedures were performed by the same technician. Flushing was carried out using PBS (Dullbecco, Nutricell®, Campinas, SP, Brazil). The recovered embryos were transferred to Petri dishes containing the manipulation medium TQC® (AB

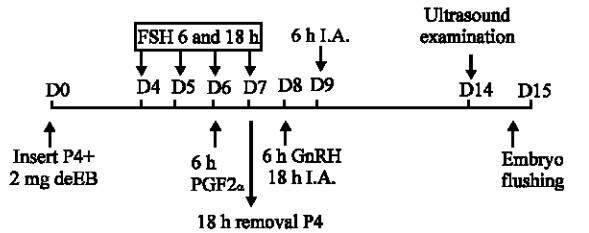

Fig. 1: Schematic representation of the protocol used for superovulation

Technology, Nutricell, Campinas, SP, Brazil). The embryos were submitted to 5 washes in the same medium and then classified morphologically under a stereomicroscope (40x). The classification by development stage (unfertilized, 2-8 cells, 8-16 cells, morulae-MO, compact morulae-CM, early blastocyst-EB, blastocyst-MB and expanded blastocyst-XB) and quality (Grade 1 or Excellent; 2 or Good; 3 or Regular 4 or Poor) was performed according to the International Embryo Transfer Society Manual (Stringfellow and Seidel, 1998). Embryos classified as Grade 1-3 embryos were considered viable and those classified as 1 and 2 as freezable Embryos.

Statistical analysis: The data were analyzed using the SAEG Statistical Software. For the normality test of the samples, the Shapiro-Wilk test was used. The data that showed no normal distribution were evaluated using the nonparametric Kruskal-Wallis test. The distribution of embryos at various stages of development and the percentage of viable and freezable embryos were evaluated by the χ^2-test. The data that were under 5 were analyzed using the Fisher's exact test. In all tests a significance level of 5% was adopted.

RESULTS AND DISCUSSION

A dose-dependent effect was observed in the number of CL and recovered structures (unfertilized oocytes and embryos; $p<0.05$) for which the 200 mg dose produced the best results. There was no significant difference ($p>0.05$) between doses of 100 and 133 mg for the same parameters mentioned above. The number of viable embryos and the number of freezable embryos did not differ ($p>0.05$) between the three groups (Table 1).

As for the stage of embryo development, the majority of recovered embryos was between the compact morulae and blastocyst stages ($p>0.05$). Structures in the stages of morulae and expanded blastocyst were also found but to a lesser quantity ($p<0.05$) (Fig. 2).

The relationship between the number of viable embryos/total number of embryos, freezable embryos/total embryos and the total number of embryos in each dose is shown in Fig. 3. The percentage of viable and freezable embryos in groups
treated with 100, 133 and 200 mg Folltropin was not significant (76 and 70, 91 and 91, 67 and 65%, respectively).

Considering the protocol in fixed time insemination, the use of FSH as an inducer of superovulation has been extensively studied to evaluate the use of different doses (Lerner *et al*., 1986; Visintin *et al*., 1999; Baruselli *et al*., 2005, Barati *et al*., 2006). It is generally, accepted that the dose required to superovulated *Bos taurus* indicus cows is smaller than that used for *Bos taurus taurus* cows (Barati *et al*., 2006).

Table 1: Mean±SEM of total CL, recovered structures, viable and freezable embryos Red Sindhicows superovulated with three different doses of FSH

FSH (mg)	Cows (n)	Corpora lutea number	Recovered strucutures	Viable embryos	Freezable embryos
100	16	3.4±0.69[a]	1.1±0.36[a]	0.81±0.31[a]	0.75±0.27[a]
133	13	3.3±0.56[a]	0.85±0.27[a]	0.77±0.28[a]	0.77±0.28[a]
200	14	6.4±0.85[b]	3.50±0.95[b]	2.40±0.66[a]	2.30±0.65[a]

[a, b]Values within column with different superscripts differ significantly ($p<0.05$)

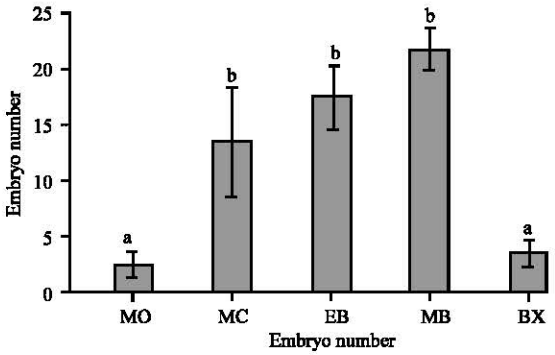

Fig. 2: Number of embryos according to stages of embryonic development of superovulated Red Sindhi cows. Morulae (MO), Compact Morulae (CM), Early Blastocyst (EB), Blastocyst (MB) and Expanded Blastocyst (XB)

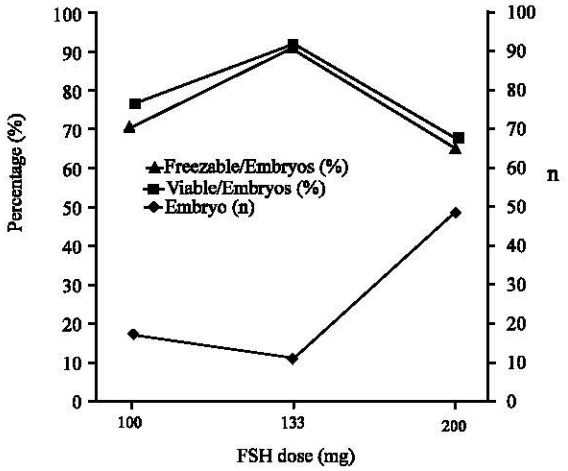

Fig. 3: Number of embryos (n), relationship viable embryos/total embryos (%) and freezable embryos/total embryos number (%) of Red Sindhi cows according to the FSH

Baruselli *et al*. (2006) compared three different doses of Folltropin-V (100, 133 and 200 mg) for superovulation of Nellore cows (*Bos taurus* indicus) and found no significant differences in the number of recovered structures and viable embryos showing that, the dose can be reduced without altering the results. Barati *et al*. (2006)

found similar results in superovulated Sistani cows (*Bos taurus* indicus), finding no effect on the number of structures recovered by comparing the doses of 120, 160 and 200 mg Folltropin-V. Moreover, the findings of this study showed that when the doses of Folltropin-V were reduced there was a significant decrease in numbers of CL and recovered structures with the best results being obtained with a dose of 200 mg FSH.

The Sindhi breed is classified as belonging to the Sub-Group 8-C (Indo-Pakistani breeds) which is represented by the zebu with convex forehead. This group also includes Deon, Sahiwal, Nimar and Gyr breeds. The Gyr breed is commonly used in Brazil because it is well adapted and due to its good milk production. Studies conducted to evaluate the superovulatory response to different doses of FSH have also been made in Gyr breed, however, the results have been below those achieved in other breeds, both zebu and taurine, similarly to what was observed in the present research.

Prado *et al.* (2007) superovulated 67 Gyr cows with 300-500 IU of Pluset (Hertape-Calier Animal Health Juatuba, MG, Brazil), obtaining 1.4, 2.9 and 1.6 viable embryos, respectively. Similar results were reported by Prado *et al.* (2007) who compared the same doses and found an average of 1.7, 3.4 and 1.7 viable embryos, respectively. These studies, together with the results obtained in the present study suggest that cows pertaining to subgroup 8-C have low response to superovulatory treatment with exogenous FSH.

In the literature there are no reports on the follicular dynamics of Sindhi. Therefore, the use of one same protocol for another breed may be inappropriate, since, there may be differences in physiology, even though, these breeds of the same genetic group (*Bos taurus indicus*). Not only the dose of FSH used could affect the the superovulatory response but also the time to start FSH application (Maia *et al*., 2003), the mechanism of synchronization of follicular wave and the time to ovulation and artificial insemination (Gonzalez *et al*., 1990).

Borges *et al*. (2003) compared the follicular dynamics of Nellore and Gyr and observed significant differences in the rate of follicular growth and size of the ovulatory follicle. These differences may affect the response to exogenous hormones used for the control and manipulation of the estrous cycle and ovulation induction which reinforces the importance of understanding the follicular dynamics of breed to determine the best time of application of each hormone.

Although, the 200 mg dose has led to a higher average production of embryos and ovarian response (Table 2), we observed a lower recovery rate (number of recovered structures/number of CL) as shown in Fig. 2. According to Cabodevila *et al*. (2001), high FSH doses may cause a reduction in the rate of recovery due to the retention of oocytes in luteinized follicles, retention of oocytes or embryos in the oviduct or to the blocking of the ability to capture oocytes by the infundibulum with their consequent fall within the abdominal cavity.

Table 2: Mean±SEM of total CL, recovered structures, viable and freezable embryos Red Sindhi cows superovulated with three different doses of FSH

FSH (mg)	Cows (n)	Corpora lutea number	Recovered strucutures	Viable embryos	Freezable embroys
100	16	3.4±0.69^a	1.1±0.36^a	0.81±0.31^a	0.75±0.27^a
133	13	3.3±0.56^a	0.85±0.27^a	0.77±0.28^a	0.77±0.28^a
200	14	6.4±0.85^b	3.50±0.95^b	2.40±0.66^a	2.30±0.65^a

ab; Means within the same column but with different superscripts differ significantly ($p<0.05$)

Embryo quality can also be influenced by the dose of FSH used. Donaldson (1984) reported a decrease in embryo quality when increasing the FSH-p dose of 28- 60 mg. In the present study, the dose of FSH used (100, 133 and 200 mg) did not significantly ($p>0.05$) influenced the percentage of viable and freezable embryos. Prado (2006) has compared superovulated Gir cows embryo quality with three doses of FSH and has found a different result, being the dose of 400 IU better than the 300 and 500 IU for the quality of embryos.

Considering that the 200 mg dose resulted in a mean number of recovery embryos three times higher than the averages obtained with doses of 100 and 133 mg and that the percentage of viable embryos in the group of 200 mg was 67%, the use of this dose allowed to obtain a greater amount of viable embryos per collection.

Concerning the stage of embryonic development in the present study, a synchronization of development of recovered structures was observed with predominance of compact morulae and blastocyst stages. This aspect may be related to induction of ovulation with the use of GnRH at the end of the superovulation protocol.

Therefore, from the data presented it is concluded that the dosage of 200 mg induced higher number of corpora lutea and total recovered structures, however, different doses of FSH tested have not influenced the number of viable and freezable embryos from Sindhi cows.

CONCLUSION

Although, much research is being carried out on Red Sindhi such experiments are focused mainly on productive aspects of these animals (Costa *et al*., 2007a, b). Research involving reproductive physiology, especially studies related to biotechnological reproduction issues like appropriate doses of FSH for superovulation and ovarian response to hormonal treatment are scarce in this breed. Therefore, the aim of this study was to evaluate the superovulatory response of Red Sindhi cows to three different doses of FSH (Folltropin-V®).

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