International Journal of Advanced Biochemistry Research

ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; SP-8(6): 19-23
www.biochemjournal.com
Received: 02-04-2024
Accepted: 09-05-2024

Shantha Vardhan M
Veterinary Gynecology and Obstetrics, College of Veterinary Science, Tirupati, Andhra Pradesh, India

Veerabramhaiah K
Veterinary Gynecology and Obstetrics, College of Veterinary Science, Tirupati, Andhra Pradesh, India

Mutha Rao M
Livestock Research Station, Guntur, Govt. of Andhra Pradesh, Andhra Pradesh, India

Siva Kumar AVN
Veterinary Physiology, College of Veterinary Science, Tirupati, Andhra Pradesh, India

Gangaraju G
Livestock Research Station, Palamaner, Govt. of Andhra Pradesh, Andhra Pradesh, India

Corresponding Author:
Shantha Vardhan M
Veterinary Gynecology and Obstetrics, College of Veterinary Science, Tirupati, Andhra Pradesh, India

Effect of FSH dose on the super ovulatory response in Punganur Cows

Shantha Vardhan M, Veerabramhaiah K, Mutha Rao M, Siva Kumar AVN and Gangaraju G

DOI: https://doi.org/10.33545/26174693.2024.v8.i6Sa.1244

Abstract
The study was aimed to evaluate the super ovulatory response in Punganur cows by using low doses of FSH wherein donor cows were treated with 160 and 200 mg of Folltropin ®V to assess the ovarian response, follicular development, total number of ovulations per animal, embryos recovered, and concentrations of estrogen at different stages of the treatment. The results indicated that 200 mg FSHp has significantly higher ovarian response with a greater number of follicles per animal, but with no significant difference in total number of ovulations, embryos recovered, and viable embryos between groups. The concentration of estrogen was significant among different treatment days within each group, but with no significant difference between groups. However, a negative correlation was observed between the concentration of estrogen on the day of initiation of superovulation with number of ovulations and embryos collected indicating an inverse relationship. In conclusion, super ovulatory response, follicular growth, and embryo recovery rate was superior with 200 mg compared to 160 mg FSH.

Keywords: Embryo, estrogen, Folltropin ® V, punganur, superovulation

Introduction
India's diverse cattle genetic resources include 43 native breeds (Srivatsava *et al.* 2019) [28] and among them Punganur exhibits exceptional traits including best reproductive efficiency and disease resistance (Veerabramhaiah *et al.* 2012) [31]. The Punganur cattle is one of the world's smallest humped cattle and its population is declining leading to genetic degradation and risk of extinction (Ramesha, 2001) [19]. In order to enhance the population advanced reproductive techniques such as superovulation and embryo transfer have been employed. However, the outcomes of superovulation procedures have been inconsistent due to differences in FSH products and dose, breed, nutritional status, seasonal effects, and age-related factors. Since the dose of FSH plays a crucial role in determining the response and due to variations in the response between *Bos Indicus* and *Bos Taurus* (Sartori *et al.* 2016) [24], the present study undertaken to assess ovarian response by using lower doses of FSH.

Material and Methods
Punganur cows (Fig. No. 1) having good characteristics maintained under standard managemental practices were selected and divided into two groups of seven donor cows. These Group I and Group II cows were superovulated with 160 and 200 mg of FSH on the 10th day of the estrous cycle (estrus-0) intramuscularly in twice daily doses at 12 h intervals for four consecutive days (35/35, 20/20, 15/15 and 10/10) and (40/40, 30/30, 20/20 and 10/10), respectively. Immediately after the exhibition of super-estrus or 12 h after the last injection of FSH, 1500 IU of Human chorionic gonadotropin (hCG) was administered to induce ovulation. All the donor cows showing super-estrus symptoms were bred at 48 and 60 h post-Prostaglandin administration.

Seven days post-breeding non-surgical embryo collection was made after assessing the superovulatory response with the help of Ultrasonography (5.0-7.5 MHz linear-array) aided ovarian examination (Fig. No. 2 & 3). Uterine flushed media was transferred to the lab for embryo isolation, evaluation and grading based on stage of development and morphological features as grade I (Excellent), grade II (Good), and grade III (Fair) (Mapletoft, 1986) [14].

High-quality embryos were equilibrated in holding medium before freezing and were moved to freezing medium and loaded into straws under a stereozoom microscope. Cryopreservation was done using a Cryobath (IMV Technologies, France) and straws were stored in LN2 container.

Blood samples were collected during FSH superovulation from day 10 to day 13 of the estrous cycle and on the days of super-estrus and embryo collection to assess estrogen concentrations. Data was analyzed using one-way ANOVA for donor superovulatory response, two-way classification for hormone concentrations and paired t-test to compare groups on specific dates with the help of SPSS 12.0.

Fig 1: Punganur cow.

Fig 2: Multiple Follicles superovulated with Folltropin V

Fig 3: Unovulated Follicles and corpora lutea superovulated with Folltropin V

Results and Discussion

Ovarian response regarding the number of follicles after superovulation in Group I and II cows was 43.10 and 54.49%, respectively, with no significant difference between groups. Superovulatory treatment of the donor with 160 mg of FSHp resulted in the development of 1.86 ± 0.40 and 1.29 ± 0.36 follicles on the right and left ovaries, respectively, and 3.14 ± 0.63 of total follicles, with 200 mg, 2.57 ± 0.30 and 2.43 ± 0.20 follicles on the right and left ovaries, respectively, and 5.00 ± 0.44 of total follicles (Table 1). There was a significant difference in the response between Groups, but the lowered response in Group I might be due to limited binding of FSH to the receptors located on granulosa cells of antral follicle (DK *et al.* 1992) [7] or failure of response to hormone due to insufficient numbers of FSH receptors in donor cows (Veerabramhaiah *et al.* 2012) [31]. These results agree with findings of Veerabramhaiah *et al.* (2012) [31] in Punganur cattle, Reddy *et al.* (2018) [22] in Sahiwal x Jersey crossbreed and Yedukondalu (2015) [34] in Ongole cattle. Contrary to the present findings, Barati *et al.* (2006) [4] did not record any significant difference in the superovulatory response between groups. These variations between two doses might be due to individual differences in the follicle population in the ovary (Mohammed *et al.* 2019) [17] and dose of Folltropin® V stimulation on antral follicles of Group II donor cows.

Further, ovarian response is related with differences in treatments such as total dose, duration and timing of treatment and the use of additional hormones in the treatment protocol (Son *et al.* 2007) [26]. Additional factors for the differences in ovarian response might also be the genetic of the animal and its environment, dietary intake (Yaakub *et al.* 1999) [33], breed (Alvarez *et al.* 2010 [2]: Tasdemir *et al.* 2011 [29]), season (Mapletoft and Bo, 2014) [13], age (Da Costa *et al.* 2001) [6], ovarian status at the time of the treatment (Bader *et al.* 2005 [3]: Durocher *et al.* 2006 [8]) and the effects of repeated superstimulations (Malhi *et al.* 2006) [12]. Though *Bos Indicus* breeds have greater sensitivity to exogenous gonadotropins, the mean lesser number of follicles in Bos *Indicus* than exotic breeds recorded in this study is akin to the findings of Baruselli *et al.* (2006) [5], Veerabramhaiah *et al.* (2012) [31] and Reddy *et al.* (2018) [22].

The overall mean number of ovulations on the right and left ovaries were 1.00 ± 0.38 and 0.86 ± 0.26, respectively, with total ovulations of 1.86 ± 0.40 per animal, In Group II were 1.57 ± 0.37, 1.14 ± 0.34 respectively, with total ovulations of 2.71 ± 0.57 per animal (Table 1). In other indigenous breeds like Ongole (Rao *et al.* 2005) [20] and Kankrej (Sahatpure and Mehta, 2004) [23] the ovulations were more than the present findings which were superovulated with 400 mg FSH. Contrary to the present findings, good response of 18 ovulations out of 21 follicles per animal was recorded by Singhal *et al.* (2017) [25]. Similar to the present study, Baruselli *et al.* (2006) [5] observed equal ovulations with lower doses of FSH during random stages of cycle. The difference in the number of ovulations between Group I and II was not significant and akin to the findings of Souza *et al.* (2018) [27], who did not find any significant difference between 200 and 400 mg FSH, but the percentage of ovulation was lower with 200 mg FSH.

There was no significant difference between two treatments with regard to the total number of ovulations and embryos recovered. Group I resulted in to recovery of 6 total embryos and 1 unfertilized ova with a mean of 0.86 ± 0.26 and 0.14 ± 0.14, respectively, per collection with overall mean percentage of embryo recovery rate of 35.71 ± 9.91, In Group II resulted in to recovery of 11 total embryos and 1 unfertilized ova with a mean of 1.57 ± 0.48 and 0.14 ± 0.14, respectively, per collection with overall mean percentage of embryo recovery rate of 47.14 ± 10.30 (Table 1).

The percentage of total recovery of embryos and ova is higher than the observations of (Karaivanov *et al.* 1990) [10] in Russian cows and of (Nilchuen *et al.* 2012) [18] in Kamphaeng Sean beef cattle. The lesser yield of good

quality embryos in 160 mg group cows might be due to the fact that the FSHp dose might not have induced satisfactory superovulation though it is well accepted that dose of FSHp in *Bos Taurus* is 30 to 50% higher than dose recommended for *Bos Indicus* cattle (Barati *et al.* 2006) [4].

In Mertolengo cattle (Da Costa, 2001) [6] fewer unfertilized ova were recovered, Souza *et al.* (2018) [27] and Guerra *et al.* (2012) [9] recorded higher unfertilized ova recovery than the present study. Similarly, the findings of (Nilchuen *et al.* 2012) [18] were akin to the present values. The greater number of ova recoveries in other studies might be due to premature or delayed ovulation and descent and also due to differences in the synchrony between ovulation and AI (Guerra *et al.* 2012) [9].

Total viable embryo production in donor cows of Group I was lesser when compared to Group II cows with no significant difference between two protocols. Similar recovery rate reported in Sahiwal by Mishra *et al.* (2002) [16] with decreasing doses of FSHp and in Iranian *Bos Indicus* by Barati *et al.* (2006) [4] with constant doses of FSHp. But in Sahiwal (Ullah *et al.* 1998) [30], Vechur (Venkatachalapathy *et al.* 2000) [32], Ongole (Kasiraj *et al.* 2000 [11]: Rao *et al.* 2005[20]) and Kankrej cows (Sahatpure and Mehta, 2004) [23] higher embryo recovery was reported with 400 mg of FSHp.

In the present study, there was no significant difference between the two treatments in the number of embryos recovered. Similar to the present study (Baruselli *et al.* 2006) [5] also did not find any difference in the embryos recovery. Contrary to this study, the yield of recovered embryos significantly varied between different FSH doses (280, 320 and 360 mg) Ali *et al.* (2012) [1]. The lesser percentage of recovery of embryos in both the groups compared to the other reports might be due to lack of egg uptake by the infundibulum due to increased size of the ovary following gonadotropin treatment in group II and lesser response observed in Group I cows in addition to premature or delayed ovulation, delayed egg or embryo descent in both the groups.

The overall mean concentrations of estrogen in cows at the time of estrus, on the day of initiation of superovulation, at super estrus and at embryo collection with 160 mg were 42.43±3.18; 40.62±2.60; 92.81±11.43 and 46.21±1.56, with 200 mg 42.53±1.70; 29.83±3.75; 117.58±11.32 and 39.90±1.27, respectively. Suggesting increased concentration at the super-estrus probably gives an indication of the number of developing estrogenized follicles, which could have turned out to be a superovulatory response (Maurya and Mathur, 2010) [15]. The difference in the concentration of estrogen between groups was not significant, but the difference between different days of treatment within Group I and Group II was significant. At the same time, the difference in estrogen concentration on the day of super-estrus and embryo collection between groups was significantly ($p<0.05$) different, while on the day of normal estrus and initiation of superovulatory treatment, difference was not significant (Table. 3).

There was a negative and significant ($p<0.05$) correlation between the concentration of estrogen on the day of initiation of superovulatory treatment with the number of ovulations and number of embryos collected in group I, In Group II there was a positive and significant ($p<0.05$) correlation between the groups. The present findings corroborate those of Reddy *et al.* (2023) [21].

Table 1: Superovulatory response and recovery rates in donor superovulated with Folltropin® V

Dose of FSHp (n=7)	Follicles		Total Follicles	Ovulations		Total Ovulations	Recovered Embryos	Recovered Embryos (%)	Recovered Ova	Total recovery (%)
	Rt ovary	Lt ovary		Rt ovary	Lt ovary					
160 mg	1.86 ±0.40	1.29 ±0.36	3.14 ±0.63	1.00 ±0.38	0.86 ±0.26	1.86 ±0.40	0.86 ±0.26	35.71 ±9.91	0.14 ±0.14	42.86 ±13.54
200 mg	2.57 ±0.30	2.43 ±0.20	5.00 ±0.44	1.57 ±0.37	1.14 ±0.34	2.71 ±0.57	1.57 ±0.48	47.14 ±10.30	0.14 ±0.14	51.90 ±12.64

Table 2: Analysis of variance of superovulatory response and recovery rates in donor Cows with Folltropin® V

Particulars	Group I	Group II	t-value
Total Follicles	3.14±0.63 ^{a}	5.00±0.44 ^{b}	-3.12
Ovulations	1.86±0.40 ^{a}	2.71±0.57 ^{a}	-1.44
Embryos	0.86±0.26 ^{a}	1.57±0.48 ^{a}	-1.99
Ova	0.14±0.14 ^{a}	0.14±0.14 ^{a}	0.00

Means bearing different superscripts (a, b) within a row differ significantly.

Table 3: Estrogen concentration on different days of superovulatory treatment with Folltropin® V

Particulars	Group 1 n=7	Group 2 n=7	t-value n=14
Estrus	42.43±3.18^{a1}	42.53±1.70^{a1}	-0.03^{NS}
Initiation of superovulation	40.62±2.60^{a1}	29.83±3.75^{a1}	1.95^{NS}
Super-estrus	92.81±11.43^{ab1}	117.58±11.32^{b2}	-3.35*
Embryo collection	46.21±1.56^{ac1}	39.90±1.27^{ab1}	3.66*
Overall n=28	55.52±3.05^{A}	57.46±3.05 ^{A}	

Means bearing different superscripts (a, b) within a column differ significantly

Means bearing different superscripts (A, B) within a row differ significantly

Means bearing different superscripts (1, 2) within a row differ significantly

Conclusion

It is concluded that superovulatory response, follicular growth and embryo recovery rate was better with 200 mg compared to 160 mg Folltropin® V in Punganur donor cows. All the collected embryos were transferred to the Embryo Biotechnology Laboratory, Palamaner and were cryopreserved by using cryobath cryopreserved.

Acknowledgement

We express our gratitude to the Department of Veterinary Gynecology and Obstetrics, College of Veterinary Science, Tirupati, and Livestock Research Station, Palamaner, for research support and scientific advice.

Conflict of Interest: All authors declare no conflict of interest.

References

1. Ali MS, Khandoker MAMY, Afroz MA, Bhuiyan AKFH. Ovarian response to different dose levels of follicle stimulating hormone (FSH) in different genotypes of Bangladeshi cattle. Asian Australas. J Anim. Sci. 2012;25(1):52.

2. Alvarez RH, Martinez AC, Pires RM. Superovulatory response of zebu cows treated with pFSH in a single subcutaneous injection followed by an additional intramuscular sub-dose 48 h later. Reprod. Domest. Anim. 2010;45:421-24.
3. Bader JF, Kojima FN, Wehrman ME, Lindsey BR, Kerley MS, Patterson DJ. Effects of prepartum lipid supplementation on FSH superstimulation and transferable embryo recovery in multiparous beef cows. Anim. Reprod. Sci. 2005;85(1-2):61-70.
4. Barati F, Niasari-Naslaji A, Bolourchi M, Sarhaddi F, Razavi K, Naghzali E, *et al*. Superovulatory response of Sistani cattle to three different doses of FSH during winter and summer. Theriogenology. 2006;66(5):1149-1155.
5. Baruselli PS, de Sá Filho MF, Martins CM, Nasser LF, Nogueira MF, Barros CM, *et al*. Superovulation and embryo transfer in Bos indicus cattle. Theriogenology. 2006;65(1):77-88.
6. Da Costa LL, e Silva JC, Silva JR. Superovulatory response, embryo quality and fertility after treatment with different gonadotrophins in native cattle. Theriogenology. 2001;56(1):65-77.
7. DK H. Superovulation with a single subcutaneous injection of Folltropin in the cow: effects of does and site of injection. Theriogenology. 1992;37:224.
8. Durocher J, Morin N, Blondin P. Effect of hormonal stimulation on bovine follicular response and oocyte developmental competence in a commercial operation. Theriogenology. 2006;65(1):102-115.
9. Guerra AG, Tribulo A, Yapura J, Singh J, Mapletoft RJ. Lengthening the super stimulatory treatment protocol increases ovarian response and number of transferable embryos in beef cows. Theriogenology. 2012;78(2):353-360.
10. Karaivanov C, Kacheva D, Petrov M, Vlahov K, Sapundjiev E. Superovulatory response of river buffalo (*Bubalus bubalis*). Theriogenology. 1990;33(2):453-464.
11. Kasiraj R, Rao MM, Reddy NR, Misra AK, Pant HC. Superovulation and embryo recovery in Ongole cows using FSH or equine chorionic gonadotrophin. Indian J. Anim. Sci. 2000, 70(3).
12. Malhi PS, Adams GP, Pierson RA, Singh J. Bovine model of reproductive aging: response to ovarian synchronization and superstimulation. Theriogenology. 2006;66(5):1257-1266.
13. Mapletoft RJ, Bó GA. Superovulation in cattle. Bovine Reproduction; c2014. p. 696-702.
14. Mapletoft RJ, Lindsell CE, Pawlyshyn V. Effects of clenbuterol, body condition and non-surgical embryo transfer equipment on pregnancy rates in bovine recipients. Theriogenology. 1986;25(1):172.
15. Maurya SK, Mathur AK. Oestrogen and progesterone concentration in Frieswal cows during superovulation. Indian J Anim. Sci. 2010;80(8):725-726.
16. Mishra OP, Khan J Ra, Awasthi MK. Estrus synchronization with cloprostenol and effect of Follitropin treatment on superovulatory response and embryo recovery in Sahiwal Cows. Indian J. Anim. Reprod. 2002;23(2):193-195.
17. Mohammed K, Darwish G, Dohreig R, Mahmoud A, Rawash Z, Nassra AK, *et al*. Impact of Type and Dose of Recombinant Human Follicle Stimulating Hormone on Embryos Production in Dairy Cows and Their Relationship with AMH Concentration. Anim. Vet. Sci. 2019;10(1):104-112.
18. Nilchuen P, Chomchai S, Rattanatabtimtong S. Superovulation with different doses of follicle stimulating hormone in Kamphaeng Saen beef cattle. J. Anim. Vet. Adv. 2012;11(5):676-680.
19. Ramesha KP. Commissioned paper in the Thematic Working Group on Domesticated Bio–Diversity, National Biodiversity Strategy and Action Plan, Min. of Environment Forestry, Government of India, 2001.
20. Rao MM, Mahesh YU, Rao KB. Periovulatory steroid hormone profile in relation to superovulatory responses in native Ongole cows. Indian J Anim. Reprod. 2005;26(2):87-90.
21. Reddy YP, Veerabramhaiah K, Krishna KM, Reddy YR. Estrogen and progesterone concentration during super ovulatory treatment and embryo collection in crossbred cows. J Pharma. Innov. 2023;12(7):285-289.
22. Reddy YV, Veerabramhaiah K, Krishna KM, Reddy YR. Superovulatory response in Sahiwal x Jersey crossbred cows with different doses of FSHp. Indian J. Anim. Reprod. 2018;39(2):61-63.
23. Sahatpure SK, Mehta VM. Total cholesterol level in relation to superovulatory response in Kankrej cows. Intas Polivet. 2004;5(2):226-228.
24. Sartori R, Gimenes LU, Monteiro Jr. PL, Melo LF, Baruselli PS, Bastos MR. Metabolic and endocrine differences between Bos taurus and Bos indicus females that impact the interaction of nutrition with reproduction. Theriogenology. 2016;86(1):32-40.
25. Singhal S, Singh N, Malik VS, Kumar A, Brar PS. A preliminary study on superstimulatory response using low dose FSH and subsequent embryo transfer in Sahiwal cattle during summer season. Indian J. Anim Reprod. 2017;38(2):37-39.
26. Son DS, Choe CY, Choi SH, Rae-Cho S, Kim HJ, Han MH, *et al*. Effect of estradiol benzoate or GnRH treatment prior to superstimulation in CIDR-treated, Korean native cows. Anim. Reprod. Sci. 2007;100(1-2):14-21.
27. Souza AH, Sartori R, Guenther JN, Caraviello D, Monson R, Wiltbank MC. Effect of semen source and dose of FSH on superovulatory response and embryo production in Holstein heifers. Anim. Reprod. 2018;4(3):70-76.
28. Srivastava AK, Patel JB, Ankuya KJ, Chauhan HD, Pawar MM, Gupta JP. Conservation of indigenous cattle breeds. J. Anim. Res. 2019;9(1):1-12.
29. Taşdemir U, Ağaoğlu AR, Kaymaz M, Karakaş K. Ovarian response and embryo yield of Angora and Kilis goats given the day 0 protocol for superovulation in the non-breeding season. Trop Anim Health Prod. 2011;43:1035-1038.
30. Ullah N, Anwar M. Superovulatory response of Sahiwal cows to follicle stimulating hormone. Indian J Anim. Sci. 1998;68:616-618.
31. Veerabramhaiah K, Rao VH, Naidu KV. Superovulatory response in endangered Punganur cows with FSHp. Tamilnadu. J Vet. Anim. Sci. 2012;8(4):241-244.
32. Venkatachalapathy RT, Jayachandran D, Venugopal VS, IYPE S. Multiple ovulation embryo transfer for

conserving Vechur cattle of Kerala. Indian J Anim. Sci. 2000, 70(8).
33. Yaakub H, O'callaghan D, Boland MP. Effect of type and quantity of concentrates on superovulation and embryo yield in beef heifers. Theriogenology. 1999;51(7):1259-1266.
34. Yedukondalu D. Effect of Lower Doses of FSH on Superovulatory Responses in Ongole Cattle. M.V.Sc thesis, Sri Venkateswara Veterinary Univ., Tirupati, India, 2015.