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Biometry of ovary and antral follicle following superovulation in Kangayam cows

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Abstract
In recent days, policy makers have opined to divert the research programmes in upstream areas of embryo transfer technology and such areas of research in the sphere was conservation of indigenous breeds of India. In order to accelerate the genetic conservation and improvement embryo biotechniques on Kangayam was undertaken. The study was conducted to determine the relationship between the antral follicular count (AFC) and superovulatory response in indigenous Kangayam cows. For the study a total of 12 Kangayam cows were used. The ovaries were examined ultrasonographically before the initiation, during the superovulatory treatment, before artificial insemination and on the day of embryo flushing. The number of small antral follicles of 3-6 mm in diameter in both the ovaries before superovulatory treatment was found to be significantly correlated with number of corpora lutea after superovulation, total number of embryos and number of transferable embryos recovered. The result of the present study indicated that the number of pre-antral follicular count could be used to predict the superovulatory response in indigenous Kangayam cows.

Keywords: Antral follicle count, superovulation, *Bos indicus*, cow, embryo yield

Introduction
The application of ultrasound includes the ability to monitor follicular characteristics, ovarian activity and aids in dominant follicle ablation thus helps to increase the superovulatory responses. Hence ultrasound was widely recognized owing to its preciseness in maintenance of optimal fertility. In this context, the antral follicle count (AFC), which reflects the population of antral follicles present in an ovary, has been indicated as an important phenotypic characteristic related to female fertility and closely correlated to the performance of *in vivo* and *in vitro* embryo production. Several studies have reported that females with a high AFC had greater embryo yields compared to those with medium and low AFC. Different research groups investigated the embryo production performance of females with different AFC. This parameter is variable among females (Burns *et al*., 2005 [1] and Morotti *et al*., 2017) [3]. It is unknown whether variation in the ovarian follicular reserve and the antral follicular count (AFC) can affect *in vitro* or *in vivo* embryo production in cattle. The present study explicates the use of AFC as a parameter to assess the vivo production of embryos, despite of consistent results and certain new challenges regarding AFC and embryo production.

Materials and Methods
For the study a total of 12 Kangayam cows between 2nd and 6th parity were selected and divided into two groups consist of 6 animals in each group. All the animals included in the study were free from all palpable abnormality. All selected Kangayam donor cows (n=12) were presynchronized with two doses of 500 µg $PGF_2\alpha$ (Cloprostenol Injection IP, *PG Estro*™, *Hester Biosciences Ltd.*, India) through intramuscular route at an interval of 11 days. At 72 hours after second dose of $PGF_2\alpha$ the animals were observed for estrus signs. Day of estrus following second $PGF_2\alpha$ injection was considered as day 0. In both group of donor cows, CIDR (*Eazi-Breed*™, *CIDR*® 1380, Zoetis India Pvt. Ltd., India) was inserted intravaginally on day 7 after estrus. 2 mg of Estradiol Benzoate Injection IP (*Pregheat*®, Virbac India Pvt Ltd., India) and 125 mg of Hydroxyprogesterone Injection IP (*Progesyn*™, Intas Pharmaceuticals Ltd., India) was injected intramuscularly at the time of CIDR insertion to all the donor cows. From day 11 (Day 0 = estrus), Group-I (n=6) Kangayam cows were

superstimulated using 200 mg of NIH-FSH-P1 (*Folltropin*®-*V*, Vetoquinol, Canada) in tapering dose. The group-II cows were superstimulated with 300 mg in tapering dose. On day 13, 500 µg of $PGF_2\alpha$ was given intramuscularly morning and evening and CIDR was removed 12 hours after second $PGF_2\alpha$ injection. After 12 hours of the last FSH injection, 20 µg of inj. GnRH (*Gynarich*®, Intas Pharmaceuticals Ltd., India) was given intramuscularly to each donor cow (Day 15 morning). All the donor cows in both the groups were artificially inseminated by frozen semen obtained from proven Kangayam bulls at 48 and 60 hours after $PGF_2\alpha$ injection. The superstimulated donor cows were flushed on the 7th day after first AI. The number of corpora lutea was counted per-rectally before flushing as well as by ultrasound after embryo flushing. Flushing was carried out as per the standard procedure (Periyannan, 2021) [5].

A real-time ultrasound scanner (MyLabX5 VET, Esaote® Asia Pacific Diagnostic Pvt. Ltd., Genova, Italy) outfitted with a liner array, 5 MHz frequency transrectal transducer was used to perform transrectal ultrasound scanning of the ovarian structures and superovulatory response in these animals during the superovulation and flushing programme noted. Ovarian size and structural changes were monitored at a) initiation of superovulatory treatment b) day of $PGF_2\alpha$ injection (3rd day of FSH) c) the day of AI and d) the day of embryo flushing. The total follicle population was recorded as appreciated by anechoic black structures, while CL with more echogenicity were recorded and measured. The ovarian size and structure was recorded. The embryo recovery rate was assessed by comparing the number of CL palpated per rectum followed by ultrasonography and number of embryos recovered and embryo quality in each donor.

The means and standard errors for all variables were calculated and presented. "Unpaired 't' test" was used to compare the responses of two different groups, nested 't' test was computed to assess the variations in the biometry of ovaries before and after treatment in two groups and spearman correlation co-efficient was used to establish a correlation between antral follicle count and superovulatory responses. Graph pad prism software version 8.0.1. Was used to analyse the data.

Table 1: Superovulatory changes (mean ± SE) in Kangayam cows

Particulars	Group I (n=6) 200 mg NIH-FSH-P1			
	Length (mm)		Width (mm)	
	Left ovary	Right ovary	Left ovary	Right ovary
Prior to SOV	19.8 ± 0.02	18.2 ± 0.01	13.6 ± 0.01	15.5 ± 0.01
Post SOV Heat	23.34 ± 0.01	21.79 ± 0.01	18.26 ± 0.02	20.41 ± 0.03
Flushing day	28.52 ± 0.02	27.64 ± 0.03	24.87 ± 0.04	23.38 ± 0.03
Particulars	**Group II (n=6) 300 mg NIH-FSH-P1**			
	Length (mm)		**Width (mm)**	
	Left ovary	**Right ovary**	**Left ovary**	**Right ovary**
Prior to SOV	17.8 ± 0.01	15.7 ± 0.01	14.9 ± 0.02	16.2 ± 0.02
Post SOV heat	21.86 ± 0.02	19.19 ± 0.03	19.41 ± 0.04	18.39 ± 0.02
Flushing day	25.34 ± 0.03	24.14 ± 0.02	22.97 ± 0.03	21.18 ± 0.03

Table 2: Mean (+ SE) of number of ovarian structures and their size during superovulation in Kangayam cows

Particulars	Group I (n=6) 200 mg NIH-FSH-P1			
	Numbers available		Average Size (cm)	
	CL	Follicle	CL	Follicle
Prior to SOV	1.00 ± 0.00	11.5 ± 0.80	1.43 ± 0.03	0.63 ± 0.01
Post SOV Heat	1.55 ± 0.02	8.87 ± 1.35	1.41 ± 0.02	0.89 ± 0.01
Flushing day	6.67 ± 0.80	0.75 ± 0.25	1.38 ± 0.01	0.99 ± 0.03
Particulars	**Group II (n=6) 300 mg NIH-FSH-P1**			
	Numbers available		**Average Size (cm)**	
	CL	**Follicle**	**CL**	**Follicle**
Prior to SOV	1.00 ± 0.00	12.5 ± 1.60	1.39 ± 0.01	0.59 ± 0.02
Post SOV Heat	1.25 ± 0.03	9.35 ± 1.28	1.35 ± 0.02	0.91 ± 0.03
Flushing day	7.83 ± 1.68	0.64 ± 0.10	1.44 ± 0.02	1.03 ± 0.02

Table 3: Correlation of AFC with superovulatory response, embryo recovery and transferable embryos

Parameters	Group I	Group II	P Value
AFC	11.5 ± 0.80	12.5 ± 1.60	0.05
SOV response	6.67 ± 0.80	7.83 ± 1.68	0.01
P Value	0.002#	0.05	
R^2 Value	0.631	0.92	
AFC	11.5 ± 0.80	12.5 ± 1.60	

p<0.05-statistically significant; *p*<0.01-statistically highly significant
NS Comparison between AFC and superovulatory response in group I

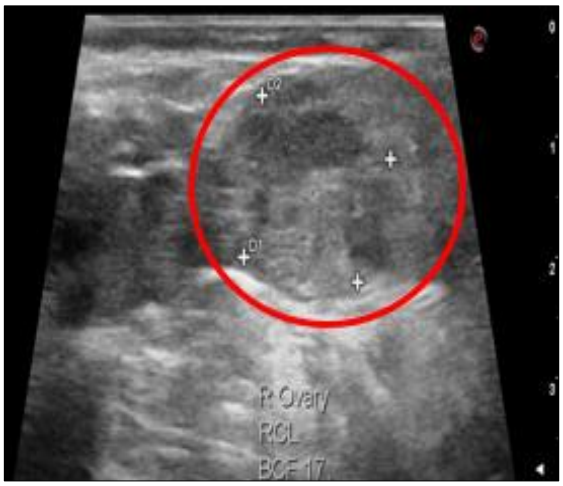

Fig 1: Biometry of RT ovary prior to start of protocol

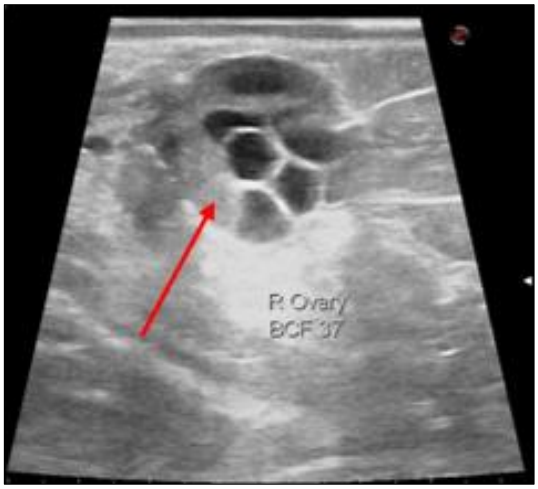

Fig 2: Multiple follicles after superovulation

Results and Discussion

The mean length and width of ovaries of Kangayam cows was recorded before and after superovulatory treatment in Kangayam cows (presented in Table. 1) and there were significant differences was observed between before and after superovulatory treatment. The biggest ovary measured was 28.52 ± 0.02 x 24.87 ± 0.04 mm in right ovary of group I and in group II (Fig.1.). Similar findings were also observed by Zangirolamo *et al*. (2018) [8]. Studies in vivo bovine model demonstrated that the ovarian dimensions significantly correlated with the antral follicle count, an outcome of superovulatory responses (U-krit *et al*., 2022) [9]. Our study has recorded an increased activity of ovarian responses in right ovary compared to left ovary and there was a significant positive correlation between mean diameter of AFC on the day of the exogenous hormonal trigger and the mean diameter of DF at the completion of the hormonal stimulation (Fig.2.); however, the Kangayam cows yielded poor ovulatory responses and AFC by virtue of its species predisposition and our findings concurred with the findings of conducted by U-krit *et al*. (2022) [9].

Despite of numerous studies aimed at computation of antral follicle population, there are many controversial data pertaining to classification of AFC exist with regards to individuals irrespective of bovine subspecies. Morotti *et al.* (2022) [9], evaluated 935 Nellore cows and generated a score card for classification of AFC as score 1, ≤ 15 follicle/ low, score 2, 16-3 follicle/ intermediate, score 3, 31-44 follicle/ high, score 4, ≥ 45 follicle very high. As per classification categorised by Morotti *et al.* (2022) [9] the Kangayam donor cows belonging to group I and group II fall under score 1 category (1 ≤ 16 follicle/ low).

Statistical analysis by Unpaired 't' test revealed that there was significant difference in number of AFC at initiation of superovulatory treatment and number of CL in between the two groups and thus the superovulatory Antral follicular count was strongly correlated with superovulatory response, embryo recovery and embryo quality (Table. 3) and revealed a highly significant positive correlation between these recorded 0 parameters (p<0.01).

Discussion

In the present study, at the time of initiation of superovulation the mean (±SE) AFC in group I and II Kangayam donor cows was 11.50±0.80 and12.50±1.60, respectively. The AFC at a given point of time reflects the ovarian follicular reserve and has been positively correlated with superovulatory responses in donor cows (Ireland *et al*., 2007 and Santos *et al*., 2016) [2, 6]. The dairy cows with low number of ovarian follicles (≤15) have lower reproductive performance as compared to cows with high number of follicles growing during follicular waves (Mossa *et al*., 2012) [4]. Hence, the AFC is a reliable predictor of the size of the ovarian reserve and positively associated with ovarian size and ovarian function. Considering the relationship between AFC and fertility characteristics, the donor selection could be performed using a single ultrasound examination at the beginning of superovulatory treatment (Zangirolamo *et al*., 2018) [8].

Conclusion

AFC can be used as an auxiliary tool for the selection of animals with the greater quantitative potential of embryos. Finally, a better understanding of the factors linked to AFC and of the reproductive characteristics from *Bos indicus* and *Bos taurus* may provide adjustments in cattle management and to improve the efficiency of reproductive bio-techniques.

The relationship between AFC and reproductive performance represents a great challenge to the current reproductive scenario in cattle for in vivo embryo production.

References

1. Burns DS, Jimenez-Krassel F, Ireland JLH, Knight PG, Ireland JJ. Numbers of antral follicles during follicular waves in cattle evidence for high variation among animals, very high repeatability in individuals, and an inverse association with serum follicle-stimulating hormone concentrations. Biol. Reprod. 2005;73:54-62.
2. Ireland JJ, Ward F, Jimenez-Krassel F, Ireland JL, Smith GW, Lonergan P. Follicle numbers are highly repeatable within individual animals but are inversely correlated with FSH concentrations and the proportion of good quality embryos after ovarian stimulation in cattle. Human Reproduction. 2007;22:1687-95.
3. Morotti F, Santos GMG, Koetz Júnior C, Silva KC, Santos KC, Roso VM, *et al*. Correlation between phenotype, genotype and antral follicle population in beef heifers. Theriogenology. 2017;91:21-26.
4. Mossa F, Walsh SW, Butler ST, Berry DP, Carter F, Lonergan P, *et al*. Low numbers of ovarian follicles≥ 3 mm in diameter are associated with low fertility in dairy

cows. Journal of dairy science. 2012;95(5):2355-2361.
5. Periyannan M. Inclusion of CIDR in superovulatory protocol on embryo recovery in Kangayam cows. M.V.Sc thesis submitted to TANUVAS, Chennai, India. 2021.
6. Santos JEP, Bisinotto RS, Ribeiro ES. Mechanisms underlying reduced fertility in anovular dairy cows. Theriogenology. 2016;86:254-262.
7. Singh J, Domínguez M, Jaiswal R, Adams GP. A simple ultrasound test to predict the superstimulatory response in cattle. Theriogenology. 2004;62(1-2):227-243.
8. Zangirolamo AF, Morotti F, Da Silva NC, Sanches TK, Seneda MM. Ovarian antral follicle populations and embryo production in cattle. Anim. Reprod. 2018;15(3):310-315.
   U-krit W, Wadsungnoen S, Yama P, Jitjumnong J, Sangkate M, Promsao N, *et al*. Understanding the Ovarian Interrelationship with Low Antral Follicle Counts (AFC) in the In Vivo Bos indicus Cow Model: Unilateral and Bilateral Main AFC as Possible Biomarkers of Ovarian Response to Hormonal Synchronisation. Biology. 2022;11(4):523. https://doi.org/10.3390/biology11040523
9. Morotti A, Sollaku I, Franceschini F, Cavazzana I, Fredi M, Sala E, De Palma G. Systematic review and meta-analysis on the association of occupational exposure to free crystalline silica and rheumatoid arthritis. Clinical Reviews in Allergy & Immunology. 2022 Apr;62(2):333-45.