Research article

DOI: 10.48165/ijar.2022.43.2.10

The Indian Journal of Animal Reproduction

The official journal of the Indian Society for Study of Animal Reproduction

Year 2022, Volume-43, Issue-2 (Dec)

ACS Publisher www.acspublisher.com

ISSN 0970-2997 *(Print)* ISSN 2583-7583 *(Online)*

Comparison of Hormonal Levels in Ovarian Follicular Fluid of Normal Versus Subfertile Buffaloes

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ABSTRACT

Six normal cycling and fifteen subfertile buffaloes were subjected to transvaginal ultrasound guided aspiration of ≥5 mm size follicles and the follicular fluid was collected on each occasion to estimate the levels of progesterone, estradiol and IGF-1. The follicles were categorized and pooled according to their size as 5 to 6, >6-8 and >8 mm. In normal cycling buffaloes, estradiol and IGF concentration increased ($P<0.05$) as the follicular size increase whereas the progesterone concentration varied non-significantly among the different size categories of follicles. In subfertile buffaloes, the concentrations of progesterone, estradiol and IGF-1 remained similar among different follicular diameter ranges. However, the concentrations of various hormones in different size categories were significantly ($P<0.05$) higher in subfertile than that in normal cycling buffaloes. In conclusion, subfertile buffaloes had unchanged concentrations of hormones among different size categories indicating disturbed steroidogenesis and require further investigations.

Key words: Estradiol, follicular fluid, IGF-I, Progesterone.

How to cite: Honparkhe, M., Gandotra, V.K., Ghuman, S.S., Matharoo, J.S., & Brar, P.S. (2022). Comparison of hormonal levels in ovarian follicular fluid of normal versus subfertile buffaloes.
The Indian Journal of Animal Reproduction, 43(2), 47–49. 10.48165/ijar.2022.43.2.10

INTRODUCTION

Follicular fluid consists serum and follicular cells' synthesized local secretions (Bellin and Ax, 1984). Changes in follicular fluid may influence steroidogenesis, oocyte developmental competence, ovulation and transport of the oocyte to the oviduct as well as the preparation of the follicle for subsequent corpus luteum formation and function (Iwata *et al*., 2006; Nicholas *et al.,* 2005). Granulosa and thecal cells of bovine follicles produce huge amounts of estrogen, progesterone and testosterone (Soboleva *et al.,* 2000). Hormonal concentrations in the follicular fluid of the bovine ovary fluctuate considerably with the stage of the cycle, follicle size and its status (Ginther *et al.,* 2001). One potential intraovarian regulator of follicular growth is insulin-like growth factor-1 (IGF-1), produced locally within the ovary and concentrations of which increase with the follicular diameter (Spicer and Echternkamp, 1995;

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Received 10-04-2023; Accepted 01-06-2023

Sharma *et al*., 2010). IGF-1 has very important role in cellular differentiation (Sirisathien *et al*., 2003; Thomas *et al.,* 2007). IGF-1 enhances glucose uptake, protein synthesis and is involved in follicular survival (Mao *et al*., 2004). The studies on hormonal level in follicular fluid have been documented in normal fertile cows and buffaloes but the same in subfertile buffaloes have not been compared. Therefore, the present study was planned to compare the follicular fluid concentrations of progesterone, estradiol 17 beta and IGF-I in normal and subfertile buffaloes.

MATERIALS AND METHODS

Six normal cycling buffaloes from university Dairy Farm and fifteen subfertile (from Private Dairy Farm, Kapurthala, Punjab) buffaloes were subjected to transvaginal ultrasound guided aspiration of ≥5 mm size follicles and the follicular fluid was collected on each occasion to estimate the levels of progesterone, estradiol and IGF-1. The follicles were categorized and pooled according to their size as 5 to 6, >6-8 and >8 mm. The ovarian follicular ablation was carried out by transvaginal ultrasound (B-mode scanner, Aloka SSD 500 equipped with 5.0 MHz sector array transducer) guided aspiration needle (Bergfelt *et al.,* 1994) (Honparkhe *et al*., 2014). Prior to the ablation, the properly restrained buffaloes were subjected to caudal epidural anaesthesia (5-10 ml of 2% Xylocaine, Inj Lignocaine, Astra Zeneca Pharma India Limited) followed by cleaning of perineal area. Individual ovary was manipulated through rectum to visualize and align the ovarian follicles to the needle guide on the ultrasound monitor. A 50 cm long sterile needle (18g) attached to a 10cc syringe was advanced through the vaginal wall into the antrum of follicle to aspirate the follicular fluid. Follicle aspiration was defined by collapse of the antral follicle following evacuation of follicular contents. This procedure was repeated for all follicles with ≥ 5 mm size in both ovaries. Follicular fluid from ablated follicles ≥ 5 mm in diameter was collected in storage vials. This fluid was stored at -20°C till hormonal analysis. Plasma progesterone was estimated through Radioimmunoassay (RIA) procedure using modified liquid phase RIA procedure (Kamboj and Prakash, 1993). The follicular estradiol- 17-beta was estimated using a commercially available Direct Immunoenzymatic Assay kit (Equipar Diagnostic Company, Italy). The levels of follicular IGF-1 were measured through Direct Immunoenzymatic Assay kit (IGF1-ELISA*, Bioline S. A-Belgium). The concentrations of various hormones in follicular fluid were statistically analyzed by t-test with unequal variance using Microsoft Office Excel 2007. A P-value of <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The concentration of different hormones in follicles of various size categories in normal and subfertile buffaloes are presented in Table 1. In normal cycling buffaloes, estradiol (2.5±2.1, 16.0±5.4 and 65.2±22.5 ng/ml in 5-6, >6-8 and >8 mm follicles, respectively) and IGF-I (5.0±3.2, 14.2±4.1 and 68.1±23.9 ng/ml in 5-6, >6-8 and >8 mm follicles, respectively) concentration increased (P<0.05) as the follicular size increased whereas the progesterone concentration varied non-significantly among the different size categories of follicles. In subfertile buffaloes, the concentrations of progesterone, estradiol and IGF-1 remained similar among different diameter ranges. However, the concentrations of various hormones in different size categories were significantly (P<0.05) higher in subfertile than that in normal cycling buffaloes (Table 1).

Figures in parentheses are the number of follicular fluid sample of corresponding size categories analysed for hormonal concentration.

In the present study, the findings in normal cycling buffaloes are in accordance with the previous study where the similar trend of estradiol increase was reported as the follicle size increased (Hooda and Yadav, 2002). The

Table 1: Mean (±SEM) concentrations of progesterone, estradiol 17 beta and IGF-1 in follicles of different size in normal cycling and subfertile buffaloes.

	Normal			Subfertile		
Hormones	**5-6 mm**	**>6-8 mm**	**>8 mm**	**5-6 mm**	**>6-8 mm**	**>8 mm**
Progesterone (ng/ml)	0.4±0.1	4.9±4.0	7.7±5.6	15.8±6.0[a]	23.6±8.3[b]	26.3±6.7[c]
	(5)	(5)	(10)	(8)	(7)	(10)
Estradiol 17-beta (ng/ml)	2.5±2.1	16.0±5.4*	53.5±39.9*	65.2±22.5[a]	59.8±29.4[b]	57.8±25.9[c]
	(5)	(4)	(8)	(6)	(7)	(10)
IGF-1 (ng/ml)	5.0±3.2	14.2±4.1*	68.1±23.9*	58.0±14.2[a]	58.0±19.1[b]	44.4±16.7[c]
	(5)	(5)	(9)	(6)	(6)	(10)

* - Significant difference (P<0.05) among different size categories in normal cycling buffaloes in a row.

[a, b, c] - Significant difference (P<0.05) of subfertile buffaloes from corresponding values in normal cycling buffaloes in a row.

increase in estradiol concentration in large follicle is associated with increased number of granulosa cells as well as enhanced aromatase activity per granulosa cell (Modina *et al.,* 2007). Intrafollicular concentrations of IGF-I are also positively correlated with follicle size and intrafollicular estradiol (Mihm *et al.,* 1997; Beg *et al.,* 2002).

Results indicated that follicular fluid progesterone concentrations were not related with differential changes in diameter or estradiol concentrations in both normal cycling and subfertile buffaloes. Similarly, earlier studies did not detect progesterone changes in follicular fluid among various size categories of follicles (Beg *et al.* 2002, Hooda and Yadav, 2002). The follicular progesterone concentration does not depend on follicular size but on degree of atresia and stage of estrous cycle (Ginther *et al*., 2001; Modina *et al.,* 2007).

CONCLUSIONS

It was concluded that in subfertile buffaloes, though the concentrations of estradiol 17-beta and IGF-1 were higher than those in normal buffaloes but the significant changes in concentrations, according to the size of follicles, were not observed which was suggestive of disturbed steroidogenesis and might be the cause of subfertility in these buffaloes which needs to be investigated more in future studies.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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