Indian Journal of Animal Sciences 64(2): 111-113, February 1994

Seasonal endocrine changes in steroid hormones of developing ovarian follicles in Surti buffaloes

A P PARMAR[1] and V M MEHTA[2]

Gujarat Agricultural University, Anand, Gujarat 388 001

Received: 29 October 1991

ABSTRACT

The effect of development of ovarian follicles and seasons on oestradiol-17 β and progesterone levels was studied in Surti buffaloes. The peak oestradiol-17 β levels were recorded in fluid collected from follicles 5-8 mm in diameter. The progesterone level in follicular fluid tended to increase with the increase in follicular diameter. Significantly lower ($P<0.01$) levels of oestradiol-17 β were recorded in all the stages of follicular development during summer. However, the progesterone level was significantly higher ($P<0.01$) in summer than in winter and monsoon.

The present study was undertaken to record the variation in oestradiol-17 β and progesterone levels in follicular fluid of developing ovarian follicles of Surti buffaloes in relation to winter, summer and monsoon seasons.

MATERIALS AND METHODS

Normal samples (210) of Surti buffalo ovaries containing only follicles but no corpora lutea were collected from local slaughterhouse during winter (November-February), summer (March-June) and monsoon (July-October). On an average 70 ovaries each were considered for individual season. The follicles were classified into 4 groups according to their diameter, viz. 1 to 4 mm (group 1), 5 to 8 mm (group 2), 9 to 12mm (group 3) and above 12 mm (group 4). Using thin needle and a syringe, the clear follicular fluid was aspirated from the base of the follicles. The volume of follicular fluids being small in 1-4 mm diameter follicles (group 1), the 5 samples each of the follicular fluid collected from this group were pooled and total 6 pooled aliquots each of follicular fluid from 1-4 mm diameter were collected for all seasons. The follicular fluid from groups 2, 3 and 4 were individually maintained. Follicular fluid samples were centrifuged at 2,500 RPM/30 min at 5°C and cell debris was discarded. The supernatants were stored at-20°C till analysis for oestradiol-17 β and progesterone levels.

Radioimmunoassay of oestradiol-17 β

The follicular fluid oestradiol-17 β was estimated by double antibody sequential radioimmunoassay as per Robertson *et al.* (1979). The free and bound forms were reported by PEG accelerated double antibody methods. The iodinated oestradiol, first and second antibodies and standard hormones were procured from Diagnostic Products Corporation, Los Angeles, USA. The cross-reactivity of antisera against oestradiol-17 β, oestradiol-17 β glucoronide, oestrone and 11 β-hydroxy testosterone was 100, 6, 0.23 and 0.002% respectively. The sensitivity of assay

* Research work sponsored by the Indian Council of Agricultural Research, New Delhi, under the All-India Co-ordinated Research Project (Endocrinology) on Buffaloes (1985-1990).

Present address: [1]Veterinary Officer, [2]Research Scientist and Head,[3]Reproductive Biology Research Unit.

was 1.4 pg/ml. The intra-assay variations were 2.7, 3.5 and 4.7%, respectively, for low, medium and high concentrations of oestradiol-17 β. The nonspecific binding was less than 1.0% and maximum binding was more than 40%.

Radioimmunoassay of progesterone

The follicular fluid progesterone content was estimated by solid phase radioimmunoassay procedure of Kubasik *et al.*(1984). The iodinated progesterone hormone kits were procured from M/s Diagnostic Products Corporation, Los Angles, USA. The cross-reactivity of anti-serum against progesterone, deoxycorticosterone, 20 dehydroprogesterone, 11-deoxy-hydroxy progesterone was 100, 1.7, 2.0 and 0.3% respectively. The sensitivity of assay was 0.5 ng/ml. The interassay and intra-assay variations were 10, 6.6, 7.2 and 8.4, 7.5, 5.8%, respectively, for low, medium and high concentrations of progesterone. The non-specific binding was less than 1% and maximum binding was above 40%.

The data collected on oestradiol-17 β and progesterone levels were analysed for variance as per the standard method of Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

The mean levels of oestradiol-17 β and progesterone in relation to follicular diameter and seasons are shown in Table 1.

The middle order follicles (5 to 8 mm diameter) had significantly higher ($P< 0.05$) oestradiol-17 β contents than smaller and larger groups of follicles. The peak oestradiol concentration recorded in follicles of 5-8 mm diameter group lay an emphasis of greater synthesis of this steroid hormone. In larger follicles, the oestradiol-17 β concentration remained lower due to greater secretion of this steroid into blood circulation or due to lower rate of its synthesis in theca cells. The peak levels of oestradiol-17 β in blood circulation on the day of oestrus when ovarian follicles are in preovulation or ovulatory stage also substantiated greater secretion of this steroid hormone in blood circulation. A similar trend was observed in mare (Short 1960), cow (Short 1962, England *et al.*1973) and sheep (Seamark *et al.*1974). The follicular fluids collected in all stages of follicular development during summer (March-June) had significantly ($P<0.01$) lower oestradiol-17 β, but during winter it was significantly higher ($P<0.05$) (Table 1). The buffaloes experience heavy heat stress during tropical summer, the ovarian

Table 1. Oestradol-17 β (ng/ml) progesterone (ng/ml) level (mean ± S E) in follicular fluid collected from developing follicles of Surti buffaloes in relation to seasons

Season	Diameter of follicles (mm) 1-6 n=6	5-8 n=6	9-12 n=6	Above 12 n=6	overall mean ± S E
			Oestradiol-17 β (ng/ml)		
Winter	17.00±3.01	78.00±7.66	16.03±3.32	4.73±1.29	28.94±16.58
Summer	7.88±1.21	10.48±2.85	23.90±4.34	1.43±0.08	10.92±4.72
Monsoon	9.90±1.47	39.17±2.99	40.0±1.44	17.30±0.33	26.59±7.65
Overall	11.59±2.71	42.35±19.58	26.64±7.06	7.52±4.84	
			Progesterone (ng/ml)		
Winter	107.33±6.36	155.00±15.22	290.00±79.31	323.00±122.9	221.41±53.73
Summer	230.00±40.52	200.00±53.09	176.67±27.84	295.00±24.36	225.41±25.63
Monsoon	111.67±14.29	130.00±7.54	135.00±18.86	148.30±24.64	131.74±7.58
Overall	149.60±40.23	161.66±20.00	200.55±46.36	256.87±56.45	

activities were much suppressed (Janakiraman and Mehta 1988). The weak symptoms of oestrus exhibited by the buffaloes during summer seem to be due to lower synthesis and secretion of oestradiol-17 β by the ovarian follicles.

The progesterone levels in follicular fluid tended to increase significantly with advancement in the development of follicles (Table 1). A wide variety of steroids including oestradiol, androgens and progesterones have been identified in follicular fluid of mare (Short 1960). The pooled samples of bovine follicular fluid contain significantly greater concentration of progesterone as compared to blood levels (Ireland *et al.*1979). This observation indicated that large amount of progesterone synthesis occurs in developing follicles and before it gets chance for diffusion in blood circulation, it is utilized as precursor for the synthesis of androgen and oestrogen. The high concentrations of progesterone in preovulatory follicles have also been reported in pigs (Eiler and Nalbandov 1977) and Rhesus monkey (Channing and Coudert 1976). The earlier studies of Seamark *et al.*(1974) showed that progesterone is synthesized in granulosa cells and is first converted to androstenedione and then to oestrogen. The androstenedione content of atretic follicles was higher in studies of Seamark *et al.* (1974). Therefore, the possible role of maintaining higher levels of progesterone in follicular fluid is to utilize it in synthesis of androstenedione in the absence of LH source (Short 1962). The rate of increase of progesterone in follicular fluid was greater in monsoon. The overall concentration of progesterone in follicular fluid was lowest in monsoon ($P<0.001$) and significantly highest in summer ($P<0.005$). The higher levels of progesterone in buffalo follicular fluid collected in summer could be related to lower ovulatory response of buffaloes. The oestradiol-17 β progesterone levels recorded in winter follicular fluid seem to be ideal for causing oocyte maturation and ovulation, and hence during this season ovulatory and fertilization responses are optimal for buffaloes of tropical countries.

It is concluded from present studies that the heat stress during summer experienced by buffaloes lower the optimal synthesis of oestradiol-17 β in ovarian follicles and thereby might lower the reproductive efficiency of these animals during summer.

REFERENCES

Channing C P and Coudert S P. 1976. Contribution of granulosa cell and follicular fluid to ovarian oestrogen secretion in the Rhesus monkey *in vivo*. *Endocrinology* **98**: 590.

Eiler H and Nalbandov A V. 1977. Sex steroid in follicular fluid and blood plasma during oestrous cycle of pig. *Endocrinology* **100**: 331.

England B G, Karavolas H J, Hauser E R and Casida L E. 1973. Ovarian follicular oestrogens in Angus heifers. *Journal of Animal Science* **37**: (5): 1176.

Ireland J J, Coulson P B and Murphree R L. 1979. Follicular development during four stages of the oestrous cycle of beef cattle. *Journal of Animal Science* **49**: 1261.

Janakiraman K and Mehta V M. 1988. *Research Bulletin on Surti Buffalo*. Reproductive Biology Research Unit, Veterinary College, Gujarat Agricultural University, Anand, Gujarat.

Kubasik N P, Hallauer C D and Broodows R G. 1984. Evaluation of direct solid phase radioimmunoassay for progesterone useful for monitoring luteal function. *Clinical Chemistry* **30** (2): 284-86.

Robertson R O, Richard H P, Peter C W and Douglas M S. 1979. Assessment of ovulation by ultrasound and plasma determination. *Obstetrics and Gynaecology* **54** (6): 686-91.

Seamark R F, Moor R M and Mc Intosh J E A.1974. Steroid hormone production by sheep ovarian follicles cultured *in vitro*. *Journal of Reproduction and Fertility* **41**: 143-58.

Short R V. 1960. Steroid present in the follicular fluid of the mare. *Journal of Endocrinology* **20**: 147-56.

Short R V. 1962. Steroid present in follicular fluid of cow. *Journal of Endocrinology* **23**: 401.

Snedecor G W and Cochran W G. 1980. *Statistical Methods*. 7th edn. Oxford and IBH Publication Co., Calcutta.