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Porcine ovarian biometry, oocyte retrieval and quality of oocytes under different seasons in north east hill region

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Pig is one of the most important domesticated livestock species of North Eastern (NE) region in India. The majority of the tribal population in the region are pork eater and thus pigs are reared by them as an integral component of farming system. The NE region represents 40.04 % (4.458 million) of total pig population (11.134 million) of India (BAHS 2010). In spite of high pig population, this region has less per capita availability of meat due to high demand in the market. The improvement of this prime species needs more attention to meet the challenges of animal protein requirement in this part of country. Pigs being devoid of sweat glands on body surface are more heat-susceptible particularly in adverse climatic conditions as high ambient temperature, high relative humidity (RH), high solar radiation and low wind speed (Davis *et al.* 2003). The heat load may induce physical, physiological and behavioural changes that can decrease production, reproduction and immune function (Finocchiaro *et al.* 2005). Proper functioning of ovarian cyclicity is most crucial for better reproduction but it is likely to be affected by varying seasons (Love 1978). Ovaries from slaughter house provide a cheap and abundant source of oocytes for *in-vitro* maturation/ fertilization of oocytes and other techniques being used in reproduction and biotechnology fields. Therefore, the present study was undertaken to study the effect of different seasons on porcine ovarian biometry, oocyte retrieval and quality of oocytes in north east hill region of India.

Porcine ovaries (1886) over a period of 12 months were collected from the local abattoirs of Shillong, Meghalaya and transported to laboratory within 1–2 h of slaughter in normal saline (NS) at 37°C supplemented with 50 µg/ml gentamicin sulphate. In the laboratory, tissue debris on the ovaries were trimmed and washed with phosphate buffer saline (PBS) solution. Morphometric study of ovaries such as weight, length and breadth of the ovaries, number of graffian follicles (GF), corpora lutea (CL) and corpora albicans (CA) were recorded. Biometry of porcine ovaries under different season's viz. monsoon (May to Sep), winter (Oct to Jan) and summer/ pre-monsoon (Feb to Apr) were calculated.

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The oocytes were collected by aspiration method using 10 ml glass syringe with 19 gauze (G) needle containing Dulbecco phosphate buffered saline (DPBS) + 0.3% bovine serum albumen (BSA) + penicillin 100 IU/ml and streptomycin 100 µg/ml. Follicular contents were placed in sterile 15 ml centrifuge tubes and allowed to settle down at 37°C in biochemical oxygen demand (BOD) incubator. The follicular content from centrifuge tubes were poured into sterile 90 mm disposable petridishes with grides and screened under stereo zoom microscope. The oocytes were picked up and washed 5 times in maturation media.

Grading of oocytes was done based on number of layers of the cumulus oocyte complexes (COC) viz. grade- I (3–5 layers of COC), grade- II (1–2 layers of COC), grade- III (partially denuded/ uneven ooplasm) and grade-IV (denuded/ degenerated).

The temperature humidity index (THI) during the study was calculated using the formula of Kelly and Bond (1971).

The collected data were subjected for statistical analysis using the Student t-test or one-way ANOVA techniques by standard methods (Snedecor and Cocharan 1994). Statistical Analysis System (SAS) with 9.2 version software was used for the analysis of data.

The morphometric parameters of porcine ovaries under different seasons are presented in Table 1. The morphological examination of porcine ovaries revealed that the shape was multi-lobulated mulberry like structure with overall average weight of 4.43 ±- 0.62 g; however there was no significant difference in weights among the seasons. The average number of GF and CL were significantly ($P<0.05$) higher in winter than monsoon but not significantly different from pre-monsoon/summer. The number of CA was significantly ($P<0.05$) higher in winter season than monsoon and summer; however there was no significant difference between monsoon and pre-monsoon/summer seasons. The recovered oocyte numbers during different seasons had same trends as earlier described for the number of observed CA per ovary.

The significantly higher ($P<0.05$) number of GF and CL

Table 1. Biometry of porcine ovaries and grading of oocytes (percentage) under different seasons in Meghalaya

Season	Month	Weight of ovary (g)	Number of GF	Number of CL	Number of CA	No. of recovered oocytes	Grading of recovered oocyte (%)			
							GradeI	Grade II	Grade III	Grade IV
Monsoon	May	5.1	5.61	3.95	4.77	3.95	16.24	56.51	18.99	8.26
	June	4.36	4.89	5.71	5.35	3.97	14.05	59.93	17.56	8.46
	July	3.73	6.58	3.79	3.32	5.89	11.72	61.37	19.90	7.01
	August	3.85	2.69	3.86	2.72	1.86	13.06	56.43	22.04	8.47
	September	3.93	2.78	5.78	3.39	2.16	15.30	59.18	19.89	5.63
	Average	4.19±0.25	4.85±1.52[a]	4.78± 0.44[a]	3.91 ± 0.49 [a]	3.56 ± 0.12[a]	14.07	58.68	19.67	7.56
Winter	October	4.19	14.6	13.68	11.98	7.56	66.19	22.64	8.53	2.64
	November	4.36	12.89	10.89	7.97	6.53	71.38	19.22	6.13	3.27
	December	5.32	10.75	9.35	5.98	5.98	69.66	21.47	6.42	2.45
	January	4.28	10.33	5.53	3.94	4.86	65.42	23.47	6.93	4.18
	Average	4.76±0.33	12.73 ±1.79[b]	9.97 ± 0.24[b]	7.89 ± 0.91[b]	6.24 ± 0.42[b]	68.16	21.70	7.00	3.13
Summer	February	5.32	12.58	10.68	5.15	6.29	17.20	39.25	37.53	6.02
	March	4.69	9.89	6.92	4.26	3.25	21.62	37.35	32.81	8.22
	April	3.75	7.51	5.78	3.47	2.12	15.35	32.58	38.35	13.72
	Average	4.46 ± 0.32	10.32 ± 0.41[b]	7.92 ± 0.27[b]	4.46 ± 0.24[a]	3.78 ± 0.19[a]	18.05	36.23	38.35	9.32
Overall average		4.43 ± 0.62	9.34 ± 2.24	7.68 ± 2.34	5.45 ± 1.68	4.52 ± 0.24	24.00	36.00	23.00	17.00

Means bearing different superscripts (a, b) within the column differ significantly ($P < 0.05$).

during winter season might be due to low temperature humidity index (THI) in this season as compared with other seasons where higher THI was reported (Table 2). High THI had direct and negative effects on ovarian physiology by inhibiting the follicular growth and oocyte quality. The present findings were in agreement with Roth *et al*. (2000) and Wolfenson *et al*. (1995), who reported heat stress reduces inhibin levels by hastening the decrease in size of the first-wave dominant follicle and the emergence of the second dominant follicle. Intra-follicular condition is important for oocyte growth and quality. The present findings were also supported by other workers who found that domestic pig exhibits a period of depressed reproductive performance during late summer and early autumn months (Bertoldo *et al*. 2009, 2010, 2011).The ovarian cyclicity has direct influence on occurrence of seasonal infertility as characterized by a reduction in farrowing rate (Love 1978), an extended weaning-to-oestrous interval (Prunier *et al*. 1994) and a delay in the onset of puberty (Paterson *et al*.1991). All these manifestations cause a considerable economic damage to the swine industry. Thus understanding the morpho-metric feature of porcine ovary could be valuable findings to manage the reproductive health of pigs during heat stress condition. The season-wise average metrological parameters, viz. ambient temperature, relative humidity and temperature humidity index of Meghalaya are presented in Table 2.

Grading of oocytes based on number of layers of cumulus oocyte complex (COC) is presented in Table 1. The quality of oocyte with grade-I was mostly (68%) in winter and remaining 18% in pre-monsoon and 14% in monsoon season. The season had significant effect on quality and gradation of oocytes and the poor quality with grade-IV was mostly observed in rainy or monsoon season, which might be due to physiological heat stress inhibiting hormonal balance and inducing malfunctioning of ovarian activities. The present findings were in agreement with Berger and Roberts (2009) and Bertoldo *et al*. (2010), who reported a disruptive effect of heat stress on the oocyte in the pig. The body temperature is an excellent indicator of an animal's susceptibility to heat load (Mader 2003). A viable alternative to using body temperature to assess animal heat load is degree of panting, respiration, or both (Gaughan *et al*. 2000).

There are several potential mechanisms by which heat stress could compromise oocytes. Heat stress was reported to alter follicular development by reducing steroid hormone production (Wolfenson *et al*. 1997, Wilson *et al*. 1998) and these changes in follicular steroid concentration could disrupt oocyte growth. In addition, heat stress reduces growth of the dominant follicle (Badinga *et al*. 1993) and causes incomplete dominance so that there is increased growth of subordinate follicles (Wolfenson *et al*.1995).

Table 2. Average temperature (^{0} F), relative humidity (RH), temperature humidity index (THI) and rainfall during different seasons in Meghalaya

Season	Temperature	(RH) %	THI	Rainfall (mm)
Monsoon	74.67 ±2.18	78.25 ±2.17	≥74	2950 ±73
Winter	59.07 ±4.06	70.16 ±3.45	66-69	590 ± 36
Pre-monsoon / summer	67.19 ±1.39	65.68 ±2.89	69-71	780 ±54

#Source: Engineering Department, ICAR Complex, Umiam (2012-13).

Incomplete dominance could result in ovulation of an aged follicle; such follicles contain oocytes with reduced competence (Mihm *et al*. 1999). In cyclic sows, anoestrus may appear mainly in summer and occasionally in winter season (Claus and Weiler 1985). Periods of infertility and late pregnancy loss are frequent in both sows and gilts during summer (Bertoldo *et al*. 2009), and periods of early pregnancy disruption exist along summer and early autumn (Tast *et al*. 2002).

It may be concluded that the number of observed follicles and recovered oocytes were higher during the winter and consequently the quality of culturable oocytes were better due to lowest stress of environmental origin. Thus, study on ovarian biometry and other parameters under varying seasonal condition could help for better reproductive management of pigs during heat stress conditions. Further, this study may be quite worthy for study on *in-vitro* maturation and fertilization of oocytes for various biotechnological studies in laboratory.

SUMMARY

The present study was conducted to evaluate the effect of different seasons on porcine ovarian biometry and centred, oocyte retrieval and quality of oocytes in north east hill region of India. Porcine ovaries (1886) were collected over a period of 12 months from local abattoirs. The morphological studies revealed mulberry shaped multi-lobulated ovaries with an average weight of 4.435 ± 0.622 g. The average number of GF and CL were significantly higher in winter than monsoon) but not significantly different from pre-monsoon/summer. The number of CA was significantly higher in winter than monsoon and summer. The number of recovered oocyte from the visible follicles in oocyte collection medium had significantly higher number in winter than other two seasons. The oocyte percentage of grade-I, grade-II, grade-III and grade-IV were found to be 24, 36, 23 and 17%, respectively. The culturable oocytes were significantly higher during winter than summer and monsoon seasons. The season had significant effect on quality and quantity of porcine ovaries which could be well utilized in-vitro maturation and fertilization by the research in field of reproduction and biotechnology.

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