estradiol-17β levels in the ovarian follicular fluid of Ankamali pigs


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ABSTRACT
A study was carried out to determine the estradiol-17β levels in the follicular fluid in relation to the size of ovarian antral follicles in Ankamali pigs. The ovaries were collected from apparently healthy, non pregnant and cyclic Ankamali pigs in the age group of 2 to 5 years which were brought for slaughtering at civil meat processing and production centre, Frazer town, Bangalore during the months of February to May, 2010. The surface antral follicles on the ovaries were classified into three groups on the basis of their diameter, viz., Group I (small, <3 mm), Group II (medium, 3-6.9 mm) and Group III (large, 7-12 mm). Follicular fluid was aspirated and pooled separately from these three groups of follicles, centrifuged and the supernatant was stored at -20 °C until used for estimation of estradiol-17β levels by enzyme linked immunosorbent assay. The results of the present study revealed that the estradiol-17β levels were significantly (P<0.05) higher in the large follicles (13.87 ± 0.32 ng/ml) compared to small follicles (11.13 ± 0.61 ng/ml). But, the estradiol-17β levels in the medium follicles (12.83 ± 0.43 ng/ml) differed non-significantly when compared with small and large follicles. It was concluded that the levels of estradiol-17β in the ovarian follicular fluid of Ankamali pig increases with the size of follicles.

Key words: Estradiol-17β, Ovarian follicular fluid, Ankamali Pigs.

INTRODUCTION
The indigenous Indian pigs are Desi, Gahuri and Ankamali, inhabiting northern India, north-eastern India and Kerala province located in southern India, respectively (Bhat et al., 1981). Ankamali pig derives its name from the Ankamali block in the Ernakulum district of Kerala. They are widely distributed in Kerala, Karnataka, Tamil Nadu, parts of Maharashtra and Andhra Pradesh. They are raised in backyard on kitchen waste and other agricultural and industrial byproducts and are maintained by poor people belonging to backward classes and tribal origin.

The components of ovarian follicular fluid indicate the growth and development of follicles (Gosden et al., 1988). Different biochemical composition of the follicular fluid in different sized follicles could be expected (Arshad et al., 2005). Estradiol is the primary estrogen which is involved in development and control of reproductive capacities and it is also required for the increased responsiveness of granulosa cells to FSH. The granulosa cells control the follicular development and large healthy follicles are characterized by high estradiol content (Hafez, 1993). The granulosa cells secrete various estrogens including the estradiol and a portion of estradiol accumulates in the follicular fluid filling the antrum. The composition of ovarian follicular fluid has been extensively studied in cow, buffaloes, sheep and to certain extent in goats. But, the work done on this aspect in pigs, particularly in Ankamali pigs is very scarce. Hence, the present study was undertaken with the objective of determining the estradiol-17β levels in the follicular fluid of different size ovarian follicles in Ankamali pigs.

MATERIALS AND METHODS
Collection of ovaries: The ovaries were collected from apparently healthy, non-pregnant and cyclic Ankamali pigs in the age group of 2 to 5 years brought...
for slaughtering at civil meat processing and production centre, Frazer Town, Bangalore during the months of February to May 2010. Immediately after collection the ovaries were placed in sterile plastic box containing 0.9 per cent sodium chloride solution and transported to the laboratory in ice cold condition within two hours. Ten ovaries were collected in each collection and such collections were repeated four times a week and thus a total of 640 ovaries were collected during the months of February to May 2010.

Collection of antral follicular fluid: In the laboratory, the ovaries were washed twice with sterile 0.9 per cent sodium chloride solution and the surface antral follicles were classified into three groups, viz., Group I (small, <3 mm), Group II (medium, 3-6.9 mm) and Group III (large, 7-12 mm) on the basis of their diameter (Kelly et al., 1988) measured using a Vernier calipers, hand lens and scale. Fluid from each follicle was aspirated using disposable sterilized insulin syringe fitted with 26 gauge needle. For each group of follicle, separate needle and syringe was used (Arshad et al., 2005). After aspiration, the follicular fluid was transferred to polythene microcentrifuge tubes. Follicular fluid from the follicles of similar size was pooled to have an adequate volume for analysis. The pooled follicular fluid samples were then subjected to refrigerated centrifugation at 1500 rpm at 5 ºC for 15 minutes to remove the blood cells, oocyte and granulosa cells. Fluid from each follicle was aspirated using disposable sterilized insulin syringe fitted with 26 gauge needle. For each group of follicle, separate needle and syringe was used (Arshad et al., 2005). After aspiration, the follicular fluid was transferred to polythene microcentrifuge tubes. Follicular fluid from the follicles of similar size was pooled to have an adequate volume for analysis. The pooled follicular fluid samples were then subjected to refrigerated centrifugation at 1500 rpm at 5 ºC for 15 minutes to remove the blood cells, oocyte and granulosa cells. The cell free follicular fluid samples were then stored at -20 ºC until used for further analysis among the 40 ovaries collected in a week. The follicular fluid aspirated was pooled as per their size. This pooling yielded two samples in each group in a week and totally 32 samples in four months.

Analysis of follicular fluid for estradiol-17β: The concentration of estradiol-17β in the follicular fluid was determined using commercially available ELISA kit procured from Aquin Technologies Pvt. Ltd, manufactured from Tigsun Diagnostics Co. Ltd, Beijing, China.

Statistical analysis: Differences between the estradiol-17β levels among the different size follicles was statistically analyzed using computerized statistical software, GraphPad Prism (San Diego, USA, 2010) by application of one-way ANOVA with Bonferroni’s post test. The values were expressed as mean ± SE. Significance or non-significance of differences between the mean values were determined at 5 per cent level of significance.

RESULTS AND DISCUSSION

The levels of estradiol-17β in the follicular fluid of three different groups of ovarian antral follicles of Ankamali pigs are presented in Table 1. The levels of estradiol-17β were significantly (P<0.05) higher in large follicles compared to small follicles. Whereas, the estradiol levels in the medium follicles differed non-significantly (P<0.05) when compared to small and large follicles throughout the period of study. As evidenced by meteorological data, February being considered as the month of transition period from winter to summer, any variation in the estradiol levels of follicular fluid was not found compared to March, April and May, the summer months.

The results of the present study corroborated with the earlier reports of Chang et al. (1976) in pigs, Henderson et al. (1982), Baszczyk et al. (2004) and Lagendijk et al. (2007) in bovines, Salem et al. (1997) and Rahman et al. (2008) in camels, Thangavel and Nayeem (2004) and Dhaware et al. (2007) in buffaloes and Tsukada et al. (2008) in mares, who reported higher levels of estradiol-17β in the follicular fluid of large follicles or preovulatory follicles compared to small follicles. As the follicle size increased there was significant increase in the content of estradiol in ovarian follicular fluid of buffaloes (Dhaware et al., 2007).

<table>
<thead>
<tr>
<th>Groups</th>
<th>February</th>
<th>March</th>
<th>April</th>
<th>May</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>11.13 ± 0.61a</td>
<td>11.42 ± 0.63a</td>
<td>11.53 ± 0.56a</td>
<td>11.87± 0.51a</td>
</tr>
<tr>
<td>Group II</td>
<td>12.83 ± 0.43ab</td>
<td>13.08 ± 0.61ab</td>
<td>12.98 ± 0.61ab</td>
<td>12.99 ± 0.49ab</td>
</tr>
<tr>
<td>Group III</td>
<td>13.87 ± 0.32b</td>
<td>14.06 ± 0.29b</td>
<td>14.24 ± 0.45b</td>
<td>13.76 ± 0.32b</td>
</tr>
</tbody>
</table>

Mean values bearing different common superscript within a column differed significantly (P<0.05).
et al., 2007) which is confirmed in the present study in Ankamali pigs. Higher levels of estradiol were found in porcine follicular fluid during winter and autumn season compared to summer and spring which was attributed to more steroidogenic activity (Stankiewicz et al. 2008) in winter and autumn.

The increase in the concentration of estradiol-17β as the follicle developed might be due to change in the environment of follicular fluid from androgenic to estrogenic secreting cells. An increase in granulosa cell numbers and/or aromatase activity could account for the decreased concentrations of androgen and increased concentrations of estradiol-17β in the follicular fluid associated with increased follicle size (Henderson et al., 1982). Thangavel and Nayeem (2004) opined that the increased levels of estradiol-17β in the large follicles might be due to response of the growing follicles to gonadotropins that induce proliferation and differentiation of both theca and granulosa cells ultimately leading to the increased ability of the follicles to produce estradiol. Ovarian follicular cells were the main site of synthesis and release of estradiol in to the follicular fluid (Rahman et al., 2008). However, Khodaei et al. (2007) reported non-significant difference in estradiol levels among small, medium and large follicles in bovines.

In the present study, it was concluded that the levels of estradiol-17β in the ovarian follicular fluid of Ankamali pigs increases with the size of the follicle which might be due to the increased number of granulosa cells and increased aromatase activity in the granulosa cells of large follicles.

REFERENCES


