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Post-Natal Dynamic Changes in Circulating Follicle-Stimulating Hormone, Luteinizing Hormone, Immunoreactive Inhibin, Progesterone, Testosterone and Estradiol-17β in Thoroughbred Colts until 6 Months of Age

Pramod DHAKAL[1, 2], Nobuo TSUNODA[3], Rie NAKAI[1, 2], Kentaro NAGAOKA[2], Yasuo NAMBO[4], Fumio SATO[5], Hiroyuki TANIYAMA[5], Gen WATANABE[1, 2] and Kazuyoshi TAYA[1, 2*]

[11]Department of Basic Veterinary Science, United Graduate School of Veterinary Sciences, Gifu University, Gifu 501-1193, [2]Laboratory of Veterinary Physiology, Department of Veterinary Medicine, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Tokyo 183-8509, [3]Shadai Corporation, Hokkaido 059-1432, [4]Hidaka Training Research Center, Japan Racing Association, Hokkaido 057-0171, [5]Department of Veterinary Pathology, Rakuno Gakuen University, Hokkaido 069-850, Japan

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The aim of present study was to clarify the post-natal profile of follicle-stimulating hormone (FSH), luteinizing hormone (LH), immunoreactive (ir)-inhibin, progesterone, testosterone, and estradiol-17β, and their relationships in Thoroughbred colts. Six hundred and thirty-six colts were used for the study. Single plasma samples from each animal were harvested from the blood drawn through jugular venipuncture. The subjects were born with high amounts of progesterone, testosterone, and estradiol-17β, all of which dropped significantly and remained at lower levels till the end of 6 months. FSH decreased transiently after birth until day 12 and then gradually increased to peak at day 100 which then maintained in lesser levels towards the end of the studied period. LH was highest during birth which decreased until day 26 and then increased slowly to sub-birth levels up to day 90. Animals were born with high amounts of ir-inhibin. It dropped slowly and halved by day 20 and then decreased towards rest of the studied period. The increase in FSH is negatively correlated with the declining ir-inhibin levels. The early increase in FSH can be the indication of early post-natal maturation of the hypothalamic pituitary testicular axis that ultimately might be responsible for priming the testes for future development.

Key words: *FSH, ir-inhibin, post-natal, steroid hormones, Thoroughbred colt*

The process of attaining puberty is a complex interplay of endocrinological factors [8]. Multi-vocal arguments ranging from 7 to 24 months have been coming regarding the pubertal age of equines [12, 17]. It has been more complex due to the fact that horses are seasonal breeders and the timing of birth influences future age for maturity. Post natal hormone patterns have been studied in cattle, sheep, and goat [19]. Gonadotropin surge in post natal male human is also evident [2]. Androgens and follicle stimulating hormone (FSH) influence the morphological differentiation of primate Sertoli cells and secretory activity of seminiferous tubules [1]. Sertoli cell number fluctuates in horses according to the season [9] and testes produces remarkable amounts of estradiol. The concentration of gonadal and pituitary hormones in fillies has been reported previously [15] from our lab. Similar study in Thoroughbred colts is lacking. Equine fetal testis at second half of gestation is the major source of circulating inhibins [22], however, it is not from maternal source as it was immunostained in interstitial cells of fetal gonad [21]. Experiments

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*Corresponding author. e-mail: taya@cc.tuat.ac.jp
P.DHAKAL and N.TSUNODA contributed equally to this study.

blocking pituitary gonadal axis during first 4 months in monkeys resulted in lower sperm counts in adulthood [2]. The remarkable phenomenon of fetal testis enlargement and its consequences on post-natal endocrinology remains unclear and the gonadal and gonadotropin hormones' characteristics in post-natal colts have been less studied. Thus, this experiment is carried out to develop further insights into the relationship of gonadal and pituitary hormones during the post natal stage of colts.

Materials and Methods

Animals

Six hundred and thirty-six Thoroughbred colts born in Hokkaido, Japan were used for the analysis of changes in FSH, luteinizing hormone (LH), immunoreactive (ir)-inhibin, progesterone, testosterone, and estradiol-17β concentrations. Out of a large herd, colts during the age of 0 day (birth) to 6 months were randomly used for blood collection. One animal was used for one time (point sampling) only. Blood samples were collected from Jugular vein into heparinized vacutainer (10 ml) during 9:00 to 12:00 hr. Plasma were harvested and stored at –20°C until assayed. Plasma samples from animals with same age were grouped under one category.

Radioimmunoassay (RIA) of FSH, LH, ir-inhibin, progesterone, testosterone, and estradiol-17β

Plasma concentration of FSH, and LH were determined by homologous double-antibody equine RIA methods as described previously [10]. Intra- and inter-assay coefficients of variation were 4.9% and 12.2% for FSH and 12.56% and 15.06% for LH, respectively.

Concentrations of progesterone, testosterone and estradiol-17β were determined by double-antibody RIA systems using ^{125}I-labeled radioligands as previously described [23]. Anti-sera against progesterone (GDN 337), testosterone (GDN 250) and estradiol-17β (GDN 244) were used in each RIA. The intra- and inter-assay coefficients of variation were 7.3% and 14.3% for progesterone, 6.3% and 7.2% for testosterone and 6.7% and 17.8% for estradiol-17β, respectively.

Plasma ir-inhibin concentrations were measured using a rabbit antiserum against purified bovine inhibin (TNDH 1) and ^{125}I-labeled 32-kDa bovine inhibin, as previously described [4]. The results were expressed in terms of 32-kDa bovine inhibin. The intra- and inter-assay coefficients of variation were 8.0% and 16.2%, respectively.

Statistical analysis

Mean value ± SEM was calculated for each hormone from each category (hours or days). One way ANOVA and Duncan's multiple range test was used to detect the significant differences in amounts of hormones in different day points at $p<0.05$ using SPSS [20] software. Bonferroni's Multiple Comparison Test and Pearson correlation at was performed using Graphpad Prism [3] software.

Results

Concentration of FSH and LH

Immediately after the birth, there were significant fluctuations in FSH levels (Fig. 1a). The plasma levels of FSH transiently decreased until day 12 after the colts were born. Although statistically not significant, day 100 had higher FSH concentrations than the rest of the days. All the days from day 30 to day 90 had higher FSH concentration than those before day 30 (Fig. 3a). The plasma level pattern of FSH was negatively correlated with that of of ir-inhibin levels ($p=0.001$, $r=-0.544$, $n=613$). Plasma LH concentrations in colts were highest during the birth (Fig. 1b, and Fig. 2b), that decreased until day 26 and then increased slowly to sub-birth levels up to day 90 (Fig. 3b). No significant changes were observed among the levels of LH throughout 180 days.

Concentration of progesterone, testosterone, estradiol-17β, and ir-inhibin

The steroid hormones, progesterone, testosterone and estradiol-17β in colts were at highest immediately after the birth (Fig. 1d, e, f). Plasma concentrations of progesterone (Fig. 1d), testosterone (Fig. 1e), and estradiol-17β (Fig. 1f) began to decline within 2 hr after the birth and significantly decreased at 12, 24, and 24 hr respectively. Their concentrations in plasma further dropped by 48 hr of birth and then maintained at lowest level throughout the 6 months of studied period (Fig. 2d, e, f ; Fig. 3d, e , f). No significant changes were noticed on those latter days. These animals were born with high amounts of ir-inhibin (Fig. 1c). Although ir-

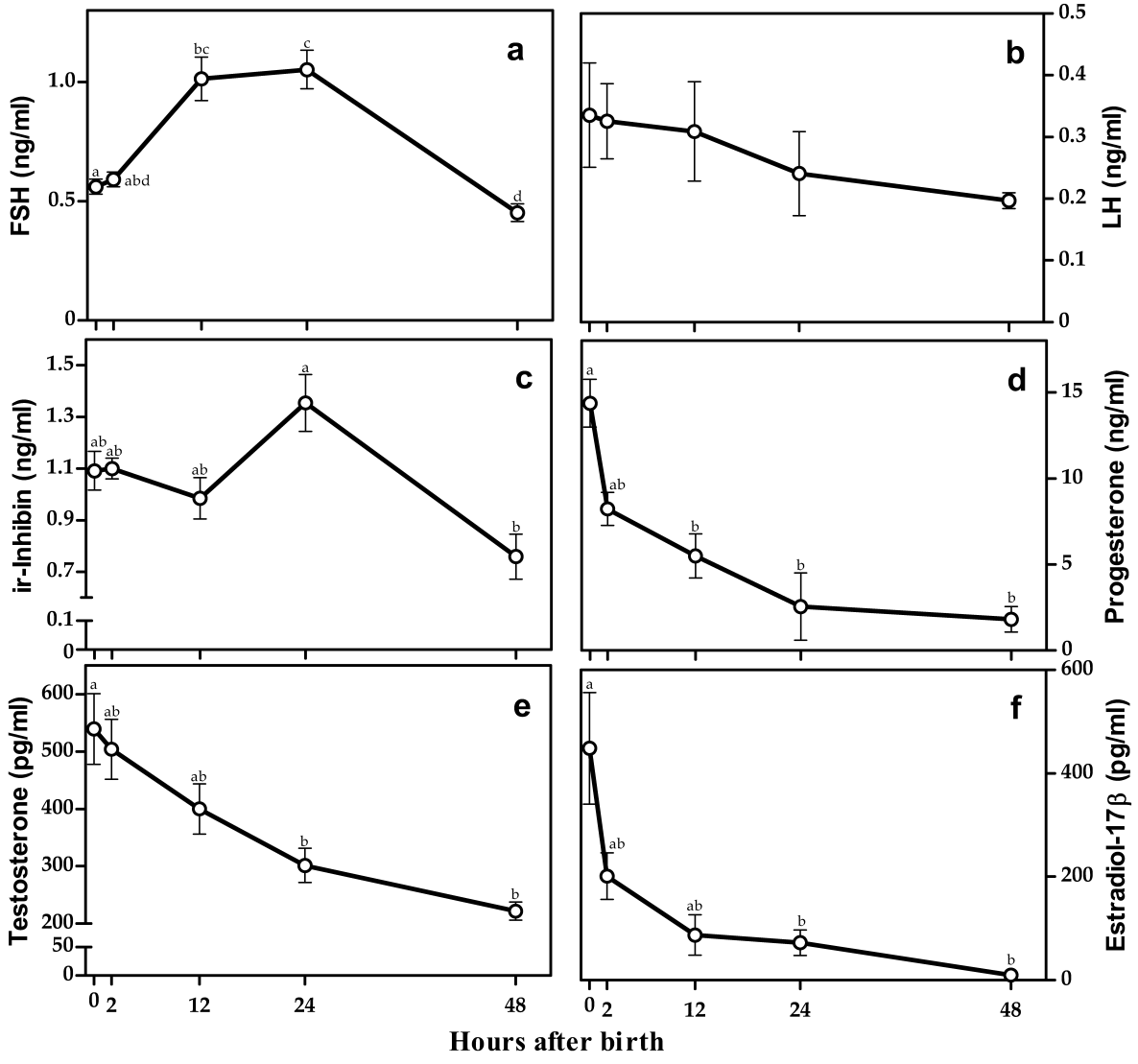

Fig. 1. Changes in circulating FSH (a), LH (b), ir-inhibin (c), progesterone (d), testosterone (e) and estradiol-17β (f) concentration (mean ± SEM) from birth to 48 hr after birth in colts.
Blood samples were collected at neonatal hour 0 (n=52), 2 (n=11), 12 (n=13), 24 (n=38), and 48 (n=17). Values with different alphabets represent significant difference.

inhibin significantly declined at 48 hr as compared with 24 hr, there was no significant decreases among 0, 2, 12, and 24 hr. Circulating ir-inhibin dropped significantly at day 5 as compared with day 1 (Fig. 2c) and the amount halved by day 20 (Fig. 3c) which then decreased slowly towards the end of studied period. Ir-inhibin was in lowest levels on day 70, 130, and 170 (Fig. 3c).

The levels of progesterone correlated significantly with testosterone (r=0.929, and n=612) and estradiol-17β (r=0.998, and n=612) at p<0.0001. Changes in plasma testosterone also significantly correlated with estradiol-17β (r=930, n=612, and p<0.0001).

Discussion

The present study demonstrated the physiological status of FSH, LH, ir-inhibin, progesterone, testosterone, and estradiol-17β in plasma of pre-pubertal

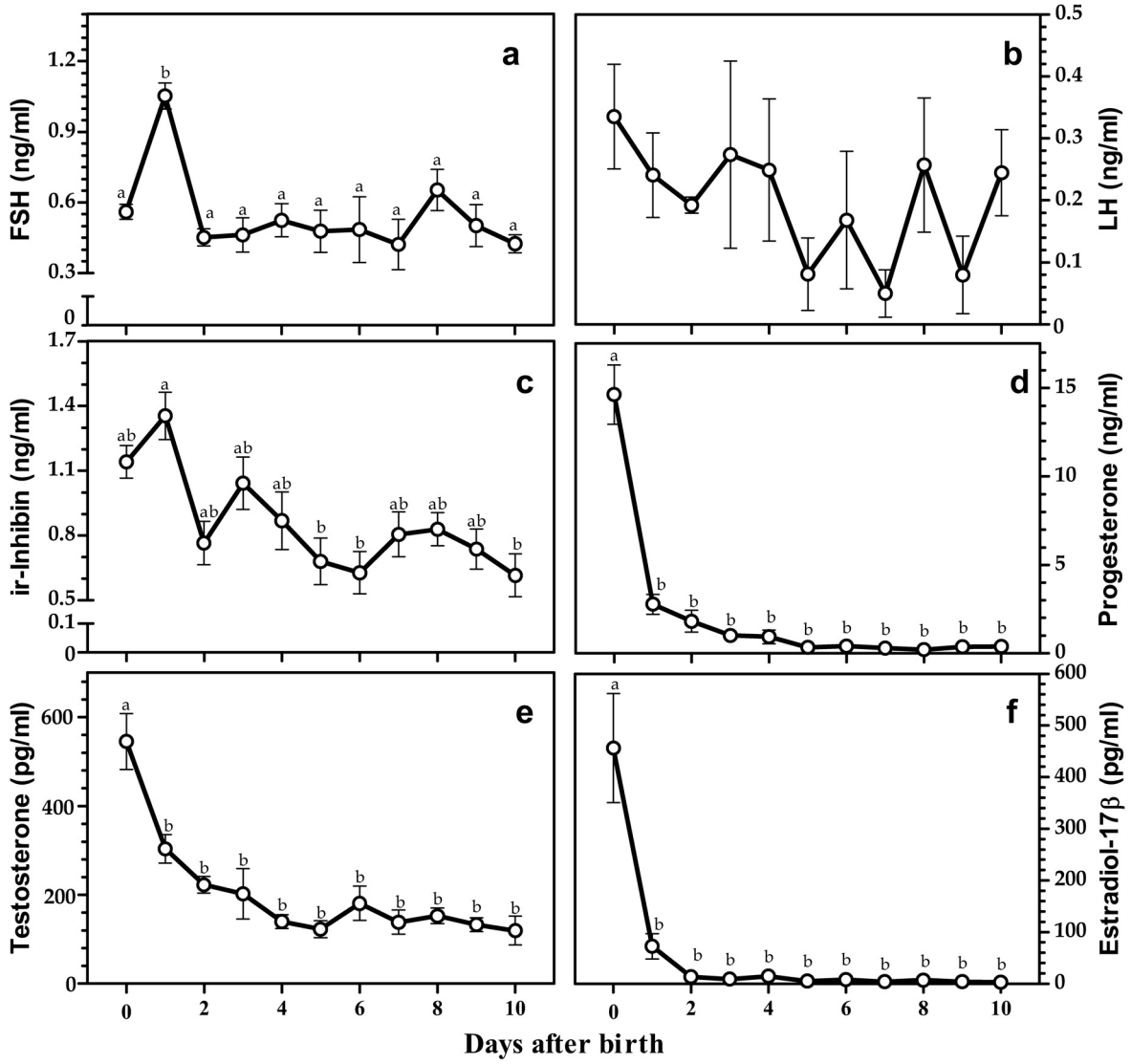

Fig. 2. Changes in circulating FSH (a), LH (b), ir-inhibin (c), progesterone (d), testosterone (e) and estradiol-17β (f) concentration (mean ± SEM) from birth to 10 days after birth in colts.
Blood samples were collected in between 9:00 to 12:00 hours on day 1 (n=38), and 2 (n=17), 3 (n=11), 4 (n=8), 5 (n=8), 6 (n=7), 7 (n=9), 8 (n=16), 9 (n=10), 10 (n=7). Blood samples were collected just after birth on day 0 (n=52). Values with different alphabets represent significant difference.

colts from immediately after birth to 6 months of age. The steroid hormones abruptly declined after the birth within days and remained at lower levels until six months. P_{450} aromatase enzyme was immunostained within the Leydig cells of 3 to 7 months old pre-pubertal colts that failed to secrete measurable quantities of estradiol in cell culture [13]. It agrees with our results where estradiol-17β is fairly lowest except for the initial high amounts at the time of birth. The high amount of steroid hormones at the time of birth is attributed to the carryover effect of their intrauterine life. Enlarged fetal testis produce high amount of Dehydro-epiandrosterone (DHEA) [18, 22], precursors for steroid hormone synthesis in feto-maternal units for androgens that decline with regression of fetal gonad. But the exact mechanism underlying this phenomenon is still unclear. In a

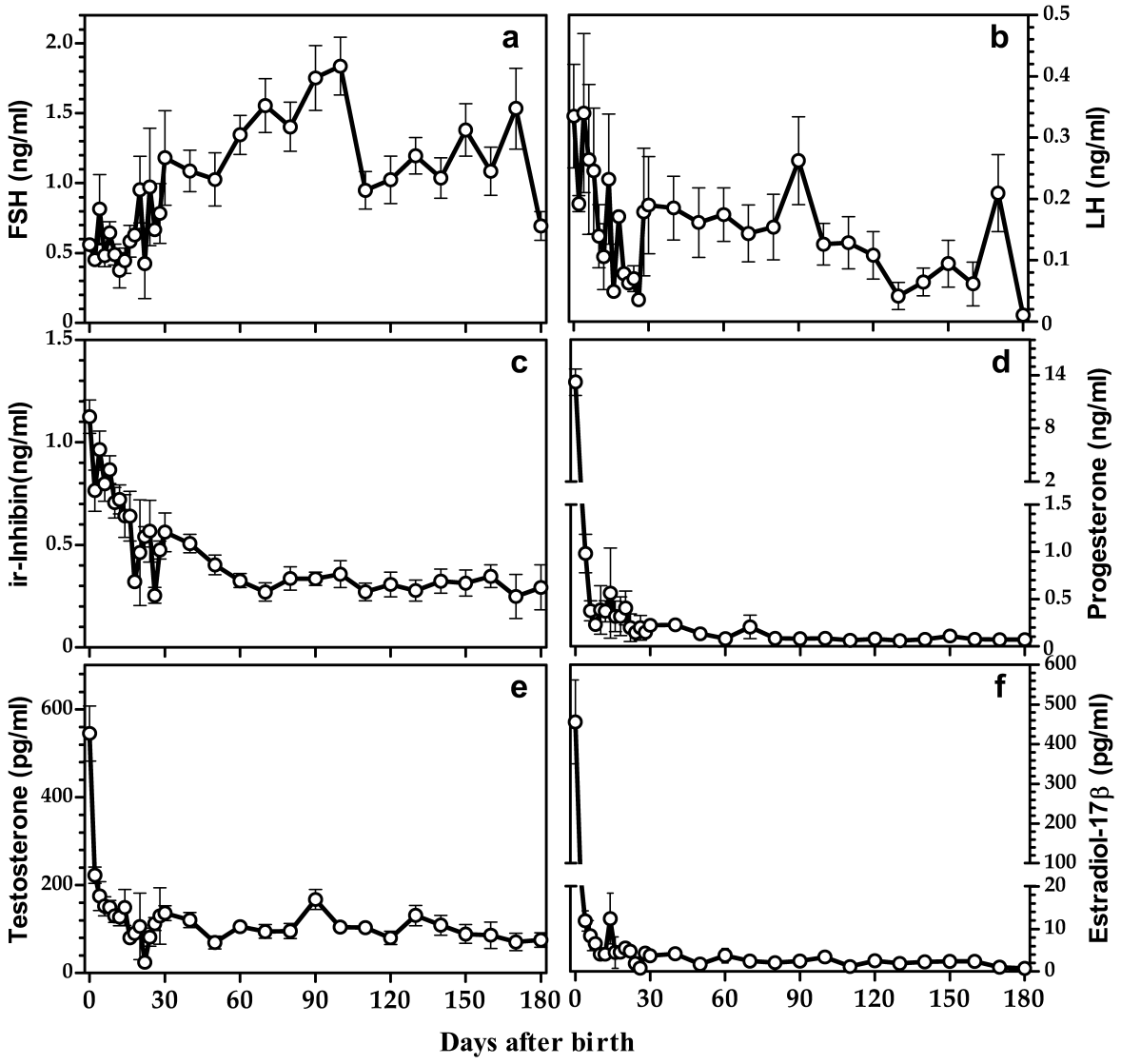

Fig. 3. Changes in circulating FSH (a), LH (b), ir-inhibin (c), progesterone (d), testosterone (e) and estradiol-17β (f) concentration (mean ± SEM) from birth to 180 days after birth in colts.
Blood samples were collected in between 9:00 to 12:00 hr on days 12 (n=9), and 14 (n=8), 16 (n=14), 18 (n=10), 20 (n=9), 22 (n=11), 24 (n=9), 26 (n=20), 28 (n=10), 30 (n=15), 40 (n=8), 50 (n=31), 60 (n=22), 70 (n=30), 80 (n=25), 90 (n=20), 100 (n=31), 110 (n=35), 120 (n=21), 130 (n=21), 140 (n=22), 150 (n=13), 160 (n=13), 170 (n=12), and 180 (n=10). Blood samples were collected just after birth on day 0 (n=52). Sample sizes from day 0 to 10 after birth are same as those shown in Fig. 2.

general picture, the rapid decline of steroid hormones can be taken as a consequence of the situation where the colts have been abruptly set free from a rich environment of these hormones inside placenta to external life.

As with the situation of the steroidogenesis process, equine fetus is capable of inhibin synthesis and unlike steroid hormones, inhibin subunits were immunostained in Sertoli cells and interstitial cells of fetal testes [22]. As the interstitial cell population in fetal testis regress towards the end of gestation, the declining levels of ir-inhibin can be logically explained. Stallions do not have stable Sertoli cell numbers and postnatal proliferation of Sertoli cells was demonstrated in black-belly sheep [5].

New studies indicate that adult Sertoli cells can be made to re-enter mitotic phase under certain experimental conditions [9]. Circulating concentrations of ir-inhibin in both male and female equine fetuses have been reported be higher than those in maternal circulation [21, 22] and similar results were found in ovine [11]. Thus, the remaining interstitial cells at neonatal stage can keep producing ir-inhibin after birth also which keeps decreasing with declining cell numbers, yet the function is taken up in reduced magnitude by proliferating Sertoli and Leydig cells with advancing age.

The levels of FSH were low at birth while ir-inhibin levels were higher. Statistically, they were negatively correlated. The negative feedback of inhibin on secretion of FSH has been demonstrated in bull calve [6, 7]. Furthermore, the high estrogens secretion by placenta may inhibit gonadotropins secretion from pituitary during gestational life of heifer [14]. This idea is supportive to our result that colts had high levels of estradiol-17β until 48 hr after birth as a consequence of intra-uterine environment thereby suppressing FSH and LH after birth. Although LH seemed higher at birth, there was great variability among animals and these levels were not significantly higher when compared with the levels at latter part of life. The increase in FSH without increase in LH in early post-natal stage of colts is indication of functionality of hypothalamo-pituitary-gonadal axis. It can be a priming action for future testicular functionality and maturity. The rise in FSH with declining ir-inhibin and no increase in gonadal steroids can be conducive for endocrine environment for the activation of the advance of spermatogenesis cascade in post-natal life. In conclusion, this study summarizes the early post-natal changes and interactions of reproductive hormones in Thoroughbred colts from immediately after birth to six months of age with possible physiological relevance during this age of life.

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