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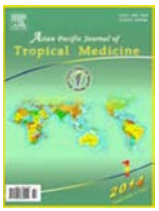

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# The effect of corpus luteum on hormonal composition of follicular fluid from different sized follicles and their relationship to serum concentrations in dairy cows

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ABSTRACT

**Objective:** To investigate the effect of the presence or absence of corpus luteum on hormonal composition of follicular fluid (FF) from different sized follicles and their relationship to serum concentrations in dairy cows.

**Methods:** Ovaries were collected from 30 clinically healthy adult female cows (Holstein Friesian) 4-7 years of age with clinically normal reproductive tracts after slaughtering. Blood samples were collected from the jugular vein before slaughter from each cow. The stage of the cycle in the cows was determined postmortem. The ovaries collected from per cow were classified with corpus luteum ($CL^+$) and without corpus luteum ($CL^-$). FF was aspirated from small (3-5 mm), medium (6-9 mm), and large (10-20 mm) follicles in $CL^+$ and $CL^-$ ovaries. Serum and FF samples were analyzed for estradiol-17β, progesterone, testosterone, $T_3$ and $T_4$ concentrations.

**Results:** Results demonstrated that the FF concentrations of estradiol-17β, progesterone and testosterone in different sized follicles categories (small, medium and large follicles in $CL^+$ and $CL^-$ ovaries) were significantly higher ($P \leqslant 0.05$) when compared with the serum. The FF concentrations of estradiol-17β and testosterone in same follicle size categories in $CL^+$ and $CL^-$ ovaries were also significant ($P<0.05$). Indeed, concentrations of these hormones in the $CL^-$ ovaries were higher than those of the $CL^+$ ovaries. However, there was a statistically significant difference between medium and large follicles for progesterone concentration in $CL^+$ and $CL^-$ ovaries ($P<0.05$). There was a significant correlation between concentration of hormones in serum and FF with increased follicular diameter.

**Conclusions:** These results indicated that the levels of hormonal composition in the FF were related to follicular size and interestingly to the presence or absence of a corpus luteum. Indeed, the corpus luteum locally affects neighboring follicular compositions during the luteal phase of the estrous cycle in dairy cows.

## 1. Introduction

Follicular fluid (FF) is an avascular compartment within the mammalian ovary, separated from the perifollicular stroma by the follicular wall that constitutes a blood-follicle barrier[1]. Besides meeting nutritional requirements of the growing oocyte, FF also maintains a proper environment for its growth and maturation[2]. This fluid is also composed of locally produced substances within the follicle, which are related to the metabolic activity of follicular cells, and it is also in part an exudate of serum[3,4]. It has been reported that FF is rich in steroid reproductive hormones including testosterone, estradiol and progesterone ($P_4$) [5]. FF and steroid hormones are commonly used in the culture media as supplements for oocyte maturation; thus, steroid concentrations in the FF need to be quantified[6]. In most mammalian species, estrogen ($E_2$) dominates the preovulatory follicular environment and meiotic division is resumed shortly before ovulation as a consequence of the preovulatory luteinizing hormone (LH) surge[7]. $P_4$ is the key hormone for maintaining pregnancy, and its production is initiated and remains low in the follicles

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prior to ovulation[7]. The androgen-$E_2$ ratio in the FF reflects the physiologic integrity and variability of the follicle. The importance of steroids, especially $P_4$, estradiol-17β and testosterone concentrations in FF has been studied by several researchers[5,8,9]. Varying degree of steroid hormonal concentration in the FF is related to size, growth of follicle, stage of estrus cycle and healthy and atretic state of the ovarian follicles[5,9-11]. In a previous study, Atheya and Totey demonstrated that the information on the concentrations of steroids hormone was important for the maturation of follicle[8]. On the other hand, Rahman *et al.* reported that the concentrations of estradiol-17β and $P_4$ increased with the growth of the follicles[12]. In addition, Yu *et al.* reported that the steroid hormone in the serum and FF was one of the major factors controlling follicular development[13].

Thyroid hormones are required for bovine ovarian follicular function[14]. Triiodothyronine ($T_3$) has been demonstrated to synergize with follicle-stimulating hormone (FSH) to induce differentiation of granulosa cells in porcine follicles[15,16] and the treatment with thyroxine ($T_4$) enhanced antral follicular development and ovulation rates in rats[17]. Blaszczyk *et al.* demonstrated that bovine FF contained the free fractions of thyroid hormones[4]. It is well known that thyroid hormones play a key role in cholesterol homoeostasis[18], and the conversion of cholesterol to pregnenolone is the first enzymatic step in the biosynthesis of all steroid hormones[13,19]. Spicer *et al.* evaluated the effects of thyroid hormones at the level of follicular cells and demonstrated that $T_3$ and $T_4$ positively contributed to LH-induced androstenedione production by bovine theca cells[20]. Additional results also suggested that $T_4$ alone had a positive impact on FSH-induced $P_4$ production by bovine granulose cells[20].

To date, reports of comparing the effect of the absence or presence of a corpus luteum (CL) on FF hormonal composition in dairy cows are limited. Corpora lutea are a continuation of follicular maturation and formed after ovulation from the remaining follicular cells. In addition, the CL is a transitory endocrine gland that secretes $P_4$, and has a key role in establishment and maintenance of pregnancy in domestic mammals[21]. In Sanjabi ewes, it has been demonstrated that the serum estradiol concentration and follicular population are mainly related to presence or absence of CL[22]. Furthermore, the number of small, medium and large follicles in ewes, with or without a CL on the ovary was significantly higher ($P<0.01$) than ewes with two ovulations at certain stages of estrous cycle[22]. On the other hand, Adams demonstrated that the absence of a dominance effect during the luteal phase of the ovine estrous cycle may be due to the suppressive effects of $P_4$ from the CL[23]. Contreras-Solis *et al.* reported that the corpora lutea affected ovarian follicular dynamics in both ovaries by a systemic effect with evidence for a local ipsilateral effect[24]. In addition, studies in small ruminants suggested a possible local effect of the CL on follicular growth[25,26]. However, currently, more information is missing regarding to the mechanisms of possible local effects of the CL on growth and hormonal composition from small to large follicles. Therefore, the aim of this study was to investigate the influence of the CL on the hormonal composition of FF from different sized follicles and their relationship to serum concentrations in dairy cows.

## 2. Materials and methods

### 2.1. Location of animals, collection of ovaries and blood

This study was performed at the Animal Reproduction Laboratory of Razi University, located in the Kermanshah province, Iran (34°18′ N and 47°3′ E) from December 2011 to February 2012 (winter). After slaughtering, the ovaries were obtained from the clinically normal reproductive tract of 30 adult cows. The stage of the cycle in the slaughtered cows was determined postmortem. About 10 mL blood samples were collected from jugular vein immediately before slaughtering from each cow. The ovaries of the cows were excised immediately after slaughter and transported to the laboratory in 0.9% normal saline, supplemented with 1 000 IU/mL penicillin G and 1 000 mg/mL streptomycin sulfate, within 1 h after slaughter. Both ovaries and the blood samples were identified using the ear tag number of the cow. Ovaries and blood samples were transported on ice (4 °C) to the laboratory.

### 2.2. Collection of FF

At the laboratory, the ovaries were again washed in saline [0.9% NaCl (4 °C)] and each ovary was cleared of the extraneous tissue. The diameter of various follicles present in each ovary was measured with a vernier calipers device. The collected ovaries were classified with ($CL^+$) and without ($CL^-$) CL. Ovaries associated with pregnant cattle and those that have any pathological lesions such as cystic follicles (>20 mm in diameter) were not included in the study. The selected follicles were separated in three different groups according to their diameter, *i.e.* small (3-6 mm), medium (6-9 mm) and large (10-20 mm). FF was aspirated from small, medium and large follicles using a sterile syringe and 22 G needle. In such cases, fluid collected from follicles of the same category from the same ovary of the same animal was pooled[27]. Follicles >20 mm in diameter with thick wall and