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ACTH administration during formation of preovulatory follicles impairs steroidogenesis and angiogenesis in association with ovulation failure in lactating cows

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ABSTRACT

Ovulation failure, follicular persistence, and formation of follicular cysts are known to impair dairy cow fertility. Although the underlying mechanism is not entirely clear, stress-induced alteration in adrenal hormone secretion can cause these ovarian pathologies. Six synchronized lactating cows were scanned daily by ultrasound, and plasma samples were taken throughout the estrous cycle. Treatment cows (n = 3) were administered with ACTH analog every 12 h from day 15 to day 21 of the cycle to induce formation of follicular cysts. Ovaries were collected at the slaughterhouse on day 23 of the cycle before appearance of follicular pathologies. Control cows (n = 3) were administered placebo, resynchronized, and administered $PGF_{2\alpha}$ on day 6 of the new cycle to induce development of a preovulatory follicle. Follicular fluid was aspirated from the preovulatory follicles of each group to determine their steroid milieu. Slices were taken from the follicular wall for total messenger (m) RNA isolation and semiquantitative reverse transcription polymerase chain reaction (RT-PCR). Administration of ACTH increased ($P < 0.02$) plasma cortisol concentration and reduced ($P < 0.01$) milk production. Androstenedione and estradiol concentrations in the follicular fluids were lower ($P < 0.05$) in ACTH-treated follicles than those in controls. The mRNA expression of luteinizing hormone receptor, 3β-hydroxysteroid dehydrogenase, cytochrome P450 aromatase ($P450_{arom}$), and cytochrome P450 17α-hydroxylase ($P450_{c17}$) were lower ($P < 0.02$) in the ACTH-treated vs control cows. On the other hand, the expression of steroidogenic acute regulatory protein and cytochrome P450 side-chain cleavage did not differ between groups. In addition, mRNA expression of vascular endothelial growth factor $(VEGF)_{120}$ and $VEGF_{164}$ was higher ($P < 0.01$) in control than in ACTH-treated follicles, but that for angiopoietin-1 and 2 did not differ between groups. Findings indicated that ACTH administration throughout preovulatory follicle development alters follicular steroidogenesis in association with impaired angiogenesis. Such alterations might explain, in part, the mechanism underlying ovulation failure and the formation of persistent or cystic follicles under stress.

1. Introduction

Development of ovarian cysts is a well-documented reproductive failure in dairy cows. Despite extensive research, the exact mechanism leading to ovulatory failure and cyst formation is not fully known. The major difficulty in studying the underlying mechanism is that cyst

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formation can only be retrospectively recognized, after the follicle has undergone extensive pathologic changes. Thus, prediction of pathology formation during follicular development is a formidable challenge [1].

In dairy cows, the postpartum period is highly susceptible, characterized by a high incidence of uterine infections [2], lameness [3], metabolic disorders [4], and negative energy balance, all associated with stress and reduced fertility. Cyst formation during the early postpartum period (from calving to 60 d postpartum) varies from 6% to 30% [1,5], presumably because of postpartum stress. For instance, lame cows are 2.6 times more likely to develop an ovarian cyst than nonlame cows [3]. Intrauterine-induced *Escherichia coli* infection, causing an increase in plasma cortisol concentration, has been found to lead to cyst formation [6].

During the follicular phase of the estrous cycle, various precisely timed events occur, which ultimately lead to ovulation of the preovulatory follicle. These include decreased progesterone and increased estradiol concentrations in the plasma which in turn increase the frequency of GnRH secretion, leading to the preovulatory LH surge and ovulation [7]. Various stresses can activate the hypothalamic–pituitary–adrenal axis, resulting in cortisol secretion from the adrenal gland [8,9]. Cortisol alters LH secretion and impairs the preovulatory LH surge, resulting in ovulatory failure [10–12]. Nevertheless, stress-induced alterations at earlier developmental stages of the preovulatory follicle have been less studied. Two processes can potentially be impaired: steroidogenesis and angiogenesis; both are enhanced during follicle development and peak at the preovulatory stage [13,14].

The aim of the present study was to assess the influence of ACTH administration on the development and functioning of the preovulatory follicle, and its association with ovulatory failure and follicle persistence. Note that all examinations were performed before final formation of a persistent follicle or cyst. We examined changes in the follicle's hormonal milieu and in the messenger RNA (mRNA) expression of genes involved in steroidogenesis and angiogenesis. The examined steroidogenic genes encoded: steroidogenic acute regulatory protein (*StAR*), cytochrome P450 side-chain cleavage ($P450_{scc}$), 3β-hydroxysteroid dehydrogenase (3β-*HSD*), cytochrome P450 17α-hydroxylase ($P450_{c17}$), cytochrome P450 aromatase ($P450_{arom}$), and LH receptor (*LHr*). The examined angiogenic genes encoded: vascular endothelial growth factor (*VEGF*), and angiopoietin-1 and 2 (*Ang*-1 and 2).

2. Material and methods

The study was carried out in the experimental dairy herd of the Agricultural Research Organization in Bet Dagan, Israel. The experiment was specifically approved by the Institutional Animal Care and Use Committee (IACUC) of the Agricultural Research Organization and the Hebrew University of Jerusalem, Israel.

2.1. Animals and treatment

Cows were milked 3 times a day, and fed a total mixed ration containing 1.7 net energy lactation Mcal/kg, 16.5% (w/w) protein, and 32% (w/w) neutral detergent fiber. Cows were equipped with electronic leg tags that allowed identification and recording of milk production 3 times a day by computer-controlled milking parlors (SAE, Afikim, Israel).

Eight cyclic multiparous Holstein cows were paired according to their days postpartum and milk production and arbitrarily assigned to control (n = 4) or treatment (ACTH; n = 4) groups. Estrus was synchronized with $PGF_{2\alpha}$ analog (500-μg Cloprostenol, Estroplan injection; Parnell Laboratories, Sydney, Australia) followed by GnRH analog (250-μg Gonadorelin, Gonabreeb injection; Parnell Laboratories) 48 h later, defined as day 0 of cycle I (Fig. 1). From day 15 to 21 of the estrous cycle, treatment cows were subjected every 12 h to subcutaneously administered ACTH analog (1-mg Tetracosactide hexaacetate, Synacthen depot; Novartis Pharma, Basel, Switzerland) as previously described by Dobson et al [9]. Cows treated with ACTH were slaughtered on day 23 of the estrous cycle.

Control cows were treated with placebo (saline). To collect the ovaries from the 2 experimental groups on the same day, estrus was resynchronized with $PGF_{2\alpha}$ injection on day 15 of cycle I followed by GnRH 48 h later, defined as day 0 of the new cycle (ie, cycle II); additional $PGF_{2\alpha}$ was administered on day 6 of cycle II, and ovaries were collected within 40 h. The second synchronization resulted in the development of a new first-wave preovulatory follicle and enabled collecting ovaries from both control and ACTH-treated cows at the same time on the same day. Finally, the ACTH-induced cysts were compared with induced first-wave preovulatory follicles.

2.2. Ultrasonography and blood sampling

Ovulations and follicular development were monitored by ultrasound machine (Aloka SSD-900, Tokyo, Japan) equipped with a 7.5-MHz transrectal linear transducer as previously described by Roth et al (2012). Ultrasound scans were performed twice a week between days 1 and 15 and every day on days 16 to 23 of the estrous cycle. Each follicle with a diameter of ≥6 mm was measured and its relative position was marked.

Blood samples were collected from the coccygeal vein or artery using EDTA vacutainer tubes (BD Vacutainer, Franklin Lakes, NJ, USA) before every ultrasound scan. Samples were centrifuged (1,150*g* for 20 min), and plasma was stored at −20°C for further analysis.

2.3. Collection of follicular fluid and wall

Ovaries were collected at a local abattoir and transferred to the laboratory in saline solution on ice within 40 min. Follicular diameter was measured and the preovulatory follicles were identified by comparing the ovarian structures with ultrasonography records. Follicular fluid was aspirated from preovulatory follicles using an 18-gauge needle connected to a 5-mL sterile syringe. Fluids were stored at −20°C for further analysis.

Samples of the follicle wall (0.25–0.5 mm^2) were collected using sterile scissors, snap-frozen in liquid nitrogen, and stored at −80°C until RNA extraction.