Theriogenology 79 (2013) 409–416

Contents lists available at SciVerse ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

# Effect of luteinizing hormone overstimulation on equine follicle maturation

S.N. Schauer [a], D. Guillaume [b], C. Decourt [b], E.D. Watson [c], C. Briant [d], F.X. Donadeu [a,*]

[a] The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush, Midlothian, UK
[b] INRA, UMR85, Physiologie de la Reproduction et des Comportements, F-37380 Nouzilly/CNRS, UMR6175, F-37380 Nouzilly/Université de Tours, Tours, France
[c] Ross University School of Veterinary Medicine, Bassterre, St. Kitts, West Indies
[d] Institut Français du cheval et de l'équitation Responsable de l'Antenne Régionale Centre Haras National de Blois, Blois, France

ARTICLE INFO

*Article history:*
Received 29 June 2012
Received in revised form 9 August 2012
Accepted 20 August 2012

*Keywords:*
LH
$PGF_{2\alpha}$
Follicular growth
Hemorrhagic anovulatory follicles
Mares

ABSTRACT

There is evidence in several species that high circulating LH concentrations can interfere with normal follicle development and ovulation. In the mare, high LH levels after induction of luteolysis with $PGF_{2\alpha}$ have been temporally associated with an increased incidence of anovulatory follicles. We hypothesized that a premature increase in LH levels during a follicular wave in mares would disrupt normal follicle maturation leading to ovulatory dysfunction. In experiment 1, all follicles >10 mm were ablated at midestrous cycle in pony mares followed by twice daily administration of equine LH (eLH; 1.6 μg/kg body weight) or saline (vehicle; N = 8 mares per group). When a dominant follicle reached >32 mm, an ovulatory dose of hCG was given. Treatment with eLH had no effects on ovulatory responses or progesterone levels during the posttreatment luteal phase. In experiment 2, after follicle ablation, mares were treated with eLH or vehicle (as above) or were given a single injection of $PGF_{2\alpha}$ (N = 7 mares per group), followed by aspiration of a dominant follicle when it reached >32 mm. Administration of eLH induced an increase in circulating LH levels similar to that after $PGF_{2\alpha}$ injection. Neither $PGF_{2\alpha}$ nor eLH administration had significant effects on follicle growth or total number of follicles in the postablation wave. However, compared with mares treated with vehicle, the preovulatory follicle in the eLH and $PGF_{2\alpha}$ groups had lower levels of androstenedione (P = 0.03) and higher levels of insulin-like growth factor I (P = 0.03). Further, levels of prostaglandin E2 in preovulatory follicles tended to be lower in the eLH and $PGF_{2\alpha}$ groups (P = 0.06). In conclusion, exposure of developing follicles to high LH in mares did not have apparent effects on ovulation but it induced changes in follicular fluid factor levels which might reflect a disruption in follicle and/or oocyte maturation, indicating the need to further study the implications of using $PGF_{2\alpha}$ for the control of fertility in farm animals.



## 1. Introduction

In monovular species such as humans and horses, fluctuations in concentrations of circulating gonadotropins stimulate the development of ovarian follicular waves and ovulation of a dominant follicle at estrus [1]. Follicular wave emergence is induced by a surge in circulating FSH levels followed by a progressive decrease in FSH as the wave continues to develop [2]. In contrast, mean circulating LH levels remain relatively low throughout the development of a wave and only distinctly increase before ovulation of a mature dominant follicle [3].

There are several lines of evidence indicating that a premature increase in circulating LH levels during follicular wave development might disrupt follicle maturation and potentially lead to ovulation failure. Transgenic mice

* Corresponding author. Tel.: +44 (0)131 651 9161; fax: +44 (0)131 651 9105.
*E-mail address:* xavier.donadeu@roslin.ed.ac.uk (F.X. Donadeu).

0093-691X/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2012.08.017

expressing a chimeric LH beta subunit displayed multiple endocrine and ovarian dysfunctions including anovulation and large hemorrhagic follicular cysts [4]. There is also a clear association between high LH, hyperandrogenism, and the formation of anovulatory cystic follicles in women with polycystic ovarian syndrome [5]. In horses, an increased incidence of ovulatory dysfunction, including premature ovulation or anovulation, and reduced fertility [6], were attributed to the relatively high LH content of pituitary preparations used to stimulate multiple ovulations [7,8]. More importantly, separate studies by Ginther and coworkers [9,10] and Cuervo-Arango and Newcombe [11] reported an association between the use of $PGF_{2\alpha}$ to artificially shorten the estrous cycle in mares and a 10-fold increase in the incidence of hemorrhagic anovulatory follicles (HAFs). The authors suggested that a premature elevation of endogenous LH levels resulting from induced luteolysis might act to disrupt or hasten follicle maturation leading to anovulation [9]. Despite the widespread use of $PGF_{2\alpha}$ as a luteolytic agent for the control of estrous cycles in farmed species and the potential implications this could have in terms of overall reproductive efficiency, the hypothesis that an increase in endogenous LH levels after administration of $PGF_{2\alpha}$ may be deleterious to follicle development has to our knowledge not been critically assessed.

The large size and long developmental time line of equine ovarian follicles, together with the accessibility of the equine ovary for precise real-time monitoring of defined follicular stages, make the mare a unique model to study the effects of artificially manipulated hormone concentrations on different aspects of follicle development. The present study tested the hypothesis that a premature elevation in LH levels during a follicular wave induced by administration of exogenous LH or administration of a luteolytic dose of $PGF_{2\alpha}$ would disrupt normal follicle maturation and that this would account, at least partially, for the recently reported effects of $PGF_{2\alpha}$-induced luteolysis on ovulation in mares [9–11].

## 2. Materials and methods

### 2.1. Experimental animals

Eight cross-bred pony mares, 3 to 14 y of age, with body weights between 250 and 425 kg and a history of good reproductive health, were kept under natural light with access to grass and haylage, water, and ad libitum mineral salts. Two different experiments were carried out during the natural breeding season (May to October) in the Northern Hemisphere (55° N, Edinburgh, UK) under the UK Home Office Animals (Scientific Procedures) Act (1986) with approval by the Ethical Review Committee, University of Edinburgh.

### 2.2. Equine LH preparation

Equine LH (eLH) was administered in the form of a highly purified pituitary fraction from crude equine gonadotropin (CEG 1.98) [8]. A dose of eLH (1.6 μg/kg body weight) that has been shown to induce ovulation in estrous mares [7] was administered iv to induce supraphysiological LH levels during follicular waves, as described below. To determine gonadotropin levels after eLH injection, in a preliminary experiment, 9 to 11 days after ovulation, mares were fitted with a jugular catheter in sterile conditions under local anesthesia and given iv 1.6 μg eLH per kg body weight or vehicle (saline, N = 4 or 5 mares per group). Blood samples were collected through the same catheter into heparinized tubes at 0, 2, 5, 15, 30, 60, 180, 360, and 720 min after eLH treatment, centrifuged at 1500× *g* for 13 min and the plasma was stored at −20 °C until hormone analyses.

### 2.3. Experimental procedures and data collection

Two different experiments were conducted sequentially to determine the effects of elevated LH levels on (1) the response of the dominant follicle to an ovulatory stimulus (experiment 1) and (2) follicle growth and the levels of follicular fluid factors associated with preovulatory maturation (experiment 2). In the second experiment, the effects of eLH administration were compared with responses to the high endogenous LH levels induced after administration of a luteolytic dose of $PGF_{2\alpha}$.

#### 2.3.1. Experiment 1. Effects of eLH administration on ovulatory responses

Ten days after a natural ovulation, all ovarian follicles >10 mm were ablated in each mare by transvaginal ultrasound-guided follicle puncture to remove atretic follicles and induce a new follicular wave, as described previously [12]. Follicles that refilled with fluid and grew to >15 mm were reablated. Mares were randomly assigned to receive eLH iv (eLH 1.6 μg/kg body weight) or an equivalent volume of vehicle (physiological saline) every 12 h (N = 8 mares per group) starting 1 day after ablation (Day 0) and continuing until a dominant follicle reached >32 mm in diameter, at which time hCG (3000 IU iv; Chorulon) was administered to induce ovulation. A crossover design was used whereby each mare was randomly assigned once to each of the two treatments leaving a complete estrous cycle between treatment periods. Throughout the treatment period, follicle growth was monitored daily by transrectal ultrasonography using a 7.5 MHz transducer on a DP-6600 Vet Digital Ultrasonic Diagnostic Imaging System (BCF Technology, Livingston, UK). At each scanning session, the diameter of follicles 10 to 15 mm was estimated by comparison with the graduation marks on the scanner screen and follicles >15 mm were measured with electronic calipers. Follicles were measured in two perpendicular planes and the average of length and width was taken as the actual diameter. After administration of hCG, the frequency of scanning was increased to every 12 h to precisely determine the time of ovulation, which was detected as per the disappearance of the previous preovulatory follicle and was confirmed by the latter presence of an echoic corpus luteum. The development of HAFs was established based on published ultrasound criteria [11]. Blood samples were collected daily into heparinized tubes immediately before the morning eLH administration and until 12 days postovulation. Samples were centrifuged at 1500 × *g* for 13 min immediately after