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The waveless model: clinical and research applications of follicular wave suppression in cattle

Modelo waveless: aplicações clínicas e na pesquisa da supressão das ondas foliculares em bovinos

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Abstract

The follicular growth waves are directly linked to the fluctuations in plasma gonadotrophins, which are controlled by the hypothalamic GnRH release pattern. Therefore, if the actions of the GnRH are inhibited or blocked, the final stages of the antral follicle growth are suppressed, resulting in an induced anestrus (a.k.a. waveless model). In the human medicine, GnRH agonists or antagonists are broadly used in the control of ovarian disfunctions, as well as in the preparation of women for assisted reproductive cycles. In cattle, a similar effect can be obtained by active immunization against GnRH. This was shown to be a viable strategy, for example, for the control of chronic cases of cystic ovarian disease in oocyte donors. However, on shall take into account the substantial individual variation on the immune response and, consequently, the lack of control of the duration of the anestrus induced. The waveless model is also very useful as a research model, once it controls the potential interference of the endogenous FSH and LH, improving the sensitivity of essays with exogenous hormones and consequently reducing the required number of replicas within studies.

Keywords: follicular growth, cystic ovarian disease, gonadotrophins, in vitro embryo production.

Resumo

O padrão de crescimento folicular em ondas está diretamente associado às flutuações nas concentrações plasmáticas de gonadotrofinas, controladas por sua vez pelo padrão de liberação de GnRH hipotalâmico. Desta forma, a inibição ou bloqueio da ação do GnRH suprime as etapas finais do crescimento folicular, resultando em anestro induzido (também chamado modelo waveless). Na medicina humana, agonistas ou antagonistas de GnRH são utilizados tanto no controle de disfunções ovarianas quanto na preparação de pacientes para procedimentos de reprodução assistida. Em bovinos, este efeito pode ser obtido pela imunização ativa contra GnRH, e mostrou-se estratégia viável, por exemplo, no controle de casos crônicos de doença ovariana cística em doadoras de oócitos. Contudo, é importante considerar a grande variação individual na resposta à imunização e consequente impossibilidade de controlar a duração do anestro induzido. O modelo waveless também é de grande utilidade na pesquisa, uma vez que elimina a potencial interferência do FSH e LH endógenos, aumentando a sensibilidade nos ensaios com hormônios exógenos e consequentemente reduzindo o número de réplicas necessárias nos estudos.

Palavras-chave*: crescimento folicular, doença ovariana cística, gonadotrofinas, produção in vitro de embriões.*

Introduction

The transition between the pre-antral and antral phases of the ovarian follicle growth is characterized by a progressive increase in sensitivity and, later on, be the dependance on gonadotrophic stimulation (van den Hurk and Zhao 2005). Therefore, follicle development after this transition is determined by the fluctuations in plasma LH and FSH concentrations (Ginther et al. 1998). The interrelationship between physiologic and endocrine events is responsible for the wave pattern of follicle growth, as observed in many species and well described in cattle (Adams 1999; Mihm et al. 2002; Aerts

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and Bols 2010). The understanding of the dynamics of follicle growth and regression during estrous cycle was key for the subsequent development of strategies for the exogenous control of reproduction, including the ovulation synchronization protocols broadly used nowadays for the timed artificial insemination and embryo transfer (TAI and TET, respectively).

One of the most common ways to synchronize follicular wave emergence is to use estradiol esters to induce a transitory reduction in endogenous FSH concentrations, mimicking the physiological events associated to dominant follicle selection (Ginther 2016). Among the flaws of this approach is the significant individual variation in the moment of wave emergence (Silva et al. 2021). Follicular wave emergence may also be induced by follicle ablation by ultrasound-guided transvaginal follicle aspiration (OPU) (Amiridis et al. 2006). This approach, however, is time consuming and requires proper equipment, besides causing some degree of ovary inflammation. Moreover, aspiration of large follicles can result in steroidogenic-active residual follicles (Viana et al. 2013).

An alternative approach to control follicular wave emergence is the transitory suppression of hypothalamic FSH release using GnRH antagonists or competitive agonists. This strategy has been used to synchronize follicle growth before superstimulation in human medicine (Yang et al. 2021), as well as to achieve contraception in cats and dogs (Gobello 2012). In cattle, treatment with GnRH lead to the suppression of follicular waves, being called the waveless model (Jimenez-Krassel et al. 2018). This is an interesting model for the study of ovarian physiology, as it prevents the potential interference of endogenous gonadotrophins. However, the use of the waveless model was limited by the high cost of the drugs required.

Our research group has been using the active immunization against GnRH, a procedure previously developed for male immunocastration (Doroteu et al. 2021), to induce anestrus by suppressing follicular waves. Besides it lower cost, the immunization Against GnRH resulted in a more effective control of follicular growth beyond 4 mm in diameter, when compared to GnRH agonists (Batista et al. 2016) or antagonists (Jimenez-Krassel et al. 2018). Here we discuss some of the potential applications of the follicular wave suppression in farm animals practice and research.

Follicular wave suppression in cows with cystic ovarian disease

Ovarian follicular cysts are an ovarian disfunction characterized by failure in ovulation and persistency of large, anovulatory follicles in the absence of a corpus luteum, resulting in anestrus (Wiltbank et al. 2002). Follicular cysts are more frequently observed in high-producing dairy herds (López-Gatius et al. 2002). When diagnosed early and properly treated, prognosis is favorable, and the conventional treatments consist of hCG or GnRH analogues injection (de Rensis et al. 2008), or insertion of progesterone implants (Gümen and Wiltbank 2005).

However, ovarian cystic disease may also develop in other situations, with different clinical signs, prognosis, and response to treatment. In cows and heifers intensively used as oocyte donors, for example, the repeated aspiration of growing follicles may alter endogenous concentrations of FSH and LH (Petyim et al. 2001), increasing follicle growth rate, the incidence of co-dominance (Viana et al. 2010). Residual follicles may also remain steroidogenically active and affect subsequent follicle dynamics (Viana et al. 2013; Ghetti et al. 2016). Moreover, other risk factors for ovarian cysts are more likely to occur in oocyte donors, such as overweight or obesity (López-Gatius 2002), and the frequent use of exogenous steroids in pre-synchronization protocols. Therefore, these donors may develop a chronic condition, with multiple cysts on both ovaries, frequently associated with mucometra (Faria et al. 2019). The ovarian cystic disease may end up refractory to conventional treatments, and has caused the precocious culling of high-value oocyte donors.

Recently, our group demonstrated that the active immunization against GnRH of oocyte donors with chronic cystic ovarian disease resulted in the regression of the cysts, as observed both by a reduction in the average follicle size and in the number of follicles above 6 mm in diameter (Viana et al. 2021). We observed also an increase in the number of small (<4 mm) follicles, even though follicular emergence after follicle ablation was slower than in cycling control cows. On the other hand, there was no effect of treatment on the mucometra score. In fact, absorption of uterine fluid requires the combined action of estradiol and progesterone (Kennedy and Armstrong 1975), and both hormones remained low during the induced anestrus. Therefore, active immunization against GnRH is an alternative to avoid the precocious culling of dedicated oocyte donors due to chronic cystic ovarian disease conditions, refractory to conventional treatments.

Follicular wave suppression in oocyte donors

The selection of a functional dominant follicle results in the atresia of the cohort of subordinated follicles, and thus affect the developmental potential of cumulus-oocyte complexes (COC) recovered by OPU and used for in vitro embryo production (IVEP) (Viana et al. 2004). The presence of luteal tissue during OPU is also not desirable, as it increase bleeding during the procedure. Therefore, suppression of follicular waves could be an alternative to the preparation of donors for COC collection.

However, the low circulation concentrations of FSH in donors undergoing induced anestrus could also potentially reduce the developmental potential of the COC recovered. To address this hypothesis, donors with previous records of chronic ovarian disease that underwent treatment by active immunization against GnRH were submitted to OPU, and results were compared with those of COC recovered from slaughterhouse ovaries (Viana et al. 2021). Later on, a second study was carried on in a commercial IVEP routine, using cyclic cows submitted or not to immunization against GnRH (Paganini Filho 2022). In both studies, there was no detrimental effects of follicular wave suppression on the number or developmental potential of the COC recovered. In fact, small (2 to 3 mm) antral follicles are sensitive, but not dependent, on FSH stimulation (van den Hurk and Zhao 2005). Thus, the reduction of plasma FSH concentration to basal values may have prevented follicle growth, but not induced immediate follicular atresia. The results with immunized cows were consistent with those obtained when follicular wave suppression was induced by GnRH agonists (Batista et al. 2016), and demonstrate that induced anestrus could be a strategy to prepare donors for OPU.

It is important to highlight, however, that active immunization against GnRH shall not be recommended for non-dedicated donors, i.e., for animals that may return to a regular reproductive management. Although the induced immunity was transitory and cows eventually resumed estrous cycle, there is a substantial variation on antibody titers among individuals (Balet et al. 2014), and on cannot predict the duration of induced anestrus. Moreover, no studies have so far evaluated long-term effects of this approach on preantral follicle recruitment from ovarian reservoir or development up to antral phase.

The waveless model for ovarian physiology research

Cows and heifers with follicular waves suppressed by active immunization against GnRH present basal FSH concentrations and no follicle growth beyond 4 mm in diameter. This is a particularly interesting research model to evaluate exogenous FSH, as it reduces potential bias due to the effect of endogenous gonadotropins. Moreover, the low coefficient of variation (CV) in follicle diameter reduces the number of experimental replicas required and allows the detection of significant differences in follicle size as low as 1 mm (Pereira 2021). The high sensitivity of the model is also useful to establish dose-response curves and to compare different sources of FSH.

Pereira et al. (2021) used the waveless model to evaluate the importance of endogenous LH during superstimulation in heifers. Follicle growth rate induced by exogenous FSH was similar between immunized and control heifers. However, the lack of endogenous LH resulted in lower average follicle diameter at the end of superstimulation, and in lower morphological quality of the COC recovered. In a second study, Pereira et al. (2022) demonstrated that the detrimental effect of the lack of endogenous LH in immunized heifers was not observed when commercial FSH formulations with high LH contamination was used. These results are consistent with the expected transition from FSH to LH dependance on larger follicles (Gomez-Leon et al. 2020), and could explain part of the inconsistencies currently observed in superovulation results.

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