SHORT COMMUNICATION

Ovarian response after a GnRH challenge in seasonally anestrous ewes

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Rubianes, E., de Castro, T., Ungerfeld, R., Meikle, A. and Rivero, A. 1997. **Ovarian response after a GnRH challenge in seasonally anestrous ewes.** Can. J. Anim. Sci. **77**: 727–730. To study the ovarian response of seasonally anestrous Corriedale ewes to a gonadotropin releasing hormone challenge, daily transrectal ultrasonography or laparotomy were performed and luteinizing hormone and progesterone profiles were determined. Of the responding ewes (8/17), three ovulated and formed a healthy corpus luteum and five formed large luteinized follicular cysts that secreted low progesterone levels for 2 to 3 d. Therefore, the study of progesterone profiles alone could lead to erroneous interpretations of the ovarian response to GnRH challenge in sheep.

Key words: GnRH, progesterone, ovary, anestrous, ultrasonography, ewes

Rubianes, E., de Castro, T., Ungerfeld, R., Meikle, A. et Rivero, A. 1997. **L'effet du GnRH sur la réponse ovariënne chez la brebis pendant l'anestrous saissoniér.** Can. J. Anim. Sci. **77**: 727–730. On a etudié la réponse ovarienne chez la brebis Corriedale en anestrous saisonnier au traitement avec GnRH. La réponse a été suivie par des sessions de ultrasonographie transrectal ou par laparotomie et les profiles de LH et progesterone ont été determinés. Parmis les brebis qui ont répondu au traitement (8/17), trois ont ovulées et formées un CL normal et cinq ont formées des grands kystes folliculaires luteïnissés secretant des bas niveaux de progesterone pendant 2 ou 3 jours. Cependant, n'utiliser que les profiles de progesterone pourrait conduire á fausses interpretations de la réponse ovarienne au traitement aves GnRH chez les ovins.

Mots clés: GnRH, progesterone, ovaire, anestrous, ultrasonographie, brebis

Early work demonstrated that the administration of a single injection of GnRH to seasonally anestrous ewes induced LH release in all animals and ovulation in the majority, but luteal function, as assessed by peripheral plasma progesterone concentrations, was absent or reduced in most of the treated animals (Haresign et al. 1975). A large proportion of ewes developed only a transient rise in progesterone concentration that was released to the formation of a short-lived CL (Garverick et al. 1992). Since GnRH treatment could provoke luteinization of large follicles without ovulation (Beck et al. 1996) determination of the progesterone profiles alone is not a sure approach to evaluate ovarian response to GnRH treatment. The objective of the present study was to examine the relationship between the ovarian response and progesterone profiles after a GnRH challenge in seasonally anestrous ewes using either direct visualization, or an ultrasonic day-to-day approach.

Two experiments were conducted in successive anestrous seasons during 1995 and 1996 (October, 35° SL) using a total of 22 multiparous Corriedale ewes (a monovular breed). Ewes weighing 35 to 40 kg were fed alfalfa hay and pellets ad libitum, housed outdoors in sheltered pens (15 m × 15 m) or indoor in box stalls (3 m × 3 m) when required. Seasonal anestrus was confirmed by the absence of behavioural estrus, the absence of a CL determined by ultrasonography and by basal progesterone levels. Ewes remained with marker vasectomized rams from 1 mo before the beginning of the experiment until the end of it and were observed twice a day for estrous behavior. The experimental treatment consisted of an i.m. injection of 10.6 µg of a synthetic GnRH analogue (buserelin acetate, Receptal, Hoechst, Uruguay) on the morning, of 10 October (day 0) in both experiments. Blood samples (10 mL) were collected once daily by jugular venipuncture for progesterone determination; from 6 d before until 5 d (exp. 1) or 15 d (exp. 2) after the GnRH challenge. Samples were permitted to clot at room temperature and centrifuged within 3 h after collection. Serum was stored at –20°C until assayed for hormones. Progesterone concentrations were estimated by a direct solid phase ^{125}I RIA method previously described (Rubianes and Ungerfeld 1993). The RIA had a sensitivity of 0.1 ng mL^{-1}; the intra-assay and inter-assay coefficients of variation were 10 and 8%, respectively. Repeated endpoints measurements were examined by split-plot analysis of variance (SAS Institute, Inc. 1989). The animals used in both experiments were handled in a manner consistent with the regulations of the Canadian Council on Animal Care.

In exp. 1, on day 5 after GnRH challenge, all animals (n = 12) were laparotomized under regional and local anesthesia

Abbreviations: CL, corpus luteum; **GnRH**, gonadotropin releasing hormone; **LH**, luteinizing hormone; **RIA**, radioimmunoassay

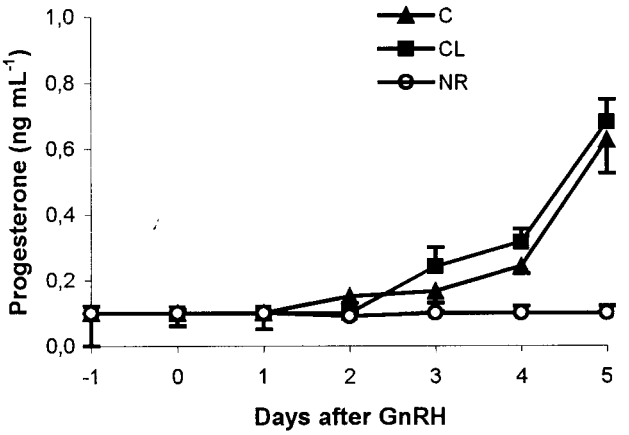

Fig. 1. Progesterone (P4) serum concentrations (mean ± SEM) of ewes with no response (NR), responding with ovulation and normal CL development (CL) or cystic formation (C), after a GnRH analogue challenge during the seasonal anestrous (exp. 1).

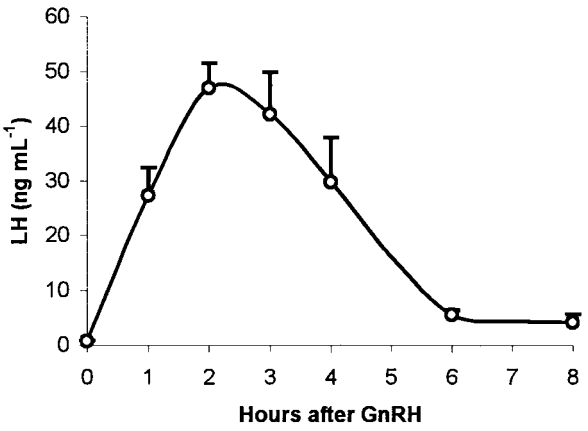

Fig. 2. Concentrations of LH (mean ± SEM) in serum of seasonal anestrous ewes treated with a GnRH analogue challenge (10.6 µg busereline acetate).

and ovaries were recovered. Number and size of the CL and of follicles greater than 1 mm in diameter present on the ovarian surface were recorded.

Experiment 2 was designed to determine ovarian dynamics assessed by ultrasonography and progesterone profiles after the GnRH challenge. The ovaries of 10 ewes were studied by transrectal ultrasonic day-to-day approach using an Aloka 210 real-time, B mode machine and a 7.5 MHz linear transducer, from 6 d before 15 d after GnRH treatment. During examination, a sketch of each ovary was made to record the diameter and position of follicles ≥3 mm in diameter. The occurrence of ovulation was indicated by the collapse of a large follicle (Rubianes et al. 1996a). To evaluate pituitary response to GnRH, additional blood samples for LH determination were taken at 0, 1, 2, 3, 4, 6 and 8 h after GnRH treatment. LH concentrations were determined in duplicate by the procedure described and validated for sheep by Forsberg et al. (1993). Inter- and intra-assay coefficients of variation were 14 and 10%, respectively. Minimum level of LH detectable was 0.2 ng mL^{-1}.

No ewe showed estrous behavior during either of the experimental periods. Retrospective analysis of progesterone profiles indicated that two ewes in exp. 1 and three ewes in exp. 2 had high progesterone concentrations (1 to 2 ng mL^{-1}) before the GnRH challenge. These ewes were excluded from further analysis since their progesterone levels indicated they were already cycling.

Direct visualization of the ovaries at time of laparotomy in exp. 1 showed that only 2 of 10 ewes had ovulated and formed a healthy CL (protruding 8 mm and 5 mm from ovarian surface, respectively). Two of the eight, non-ovulating ewes had large follicles with a thick, luteinized wall; one ewe had two (15 and 12 mm in diameter) and the other had one (10 mm in diameter). These structures were classified as luteinized follicular cysts (Murdoch 1985). None of the other six non-ovulating ewes had follicles larger than 5 mm (number of total follicles was 16.2 ± 1.5, mean ± SEM), and their progesterone levels remained low throughout the experiment. Ovulating and cystic ewes showed similar progesterone profiles that were significantly higher ($P < 0.05$) than those of the six non-ovulating ewes between days 3 and 5 after treatment (Fig. 1).

Results of exp. 2 indicate that the GnRH challenge elicited an LH surge in all ewes (Fig. 2). Basal LH concentration just before GnRH challenge averaged 0.6 ± 0.2 ng mL^{-1} whereas the mean maximum LH concentration induced was 58.1 ± 5.4 ng mL^{-1} (range: 43.4 to 79.5 ng mL^{-1}), which occurred 2 to 3 h after GnRH injection. The duration of the LH surge, as estimated from the length of time serum LH concentrations were above 2 ng mL–1 (Rawlings and Cook 1993), was at least 7 h in all ewes. Ultrasonic study showed that only one ewe had ovulated (Fig. 3a) and a CL was observed between days 5 and 14 after GnRH treatment in this ewe. In support of the ultrasound observations, this ewe showed normal luteal progesterone levels that ranged from 0.7 to 3.3 ng mL^{-1} for 10 d starting 4 d after GnRH injection. Three of the six non-ovulating ewes showed non-significant rises (= 0.4 ng mL^{-1}) in progesterone concentrations of only 1 d (Fig. 3b). In the other three non-ovulating ewes the large follicle present at time of GnRH challenge increased in size reaching a maximum diameter of 18 mm after 4 to 5 d. These structures had a non-echogenic (fluid-filled) cavity that was 9 to 14 mm in diameter with a thick luteinized wall. These cysts then regressed and at day 9 post-GnRH their diameter ranged from 5 to 7 mm. Progesterone profiles of these ewes correlated with cyst diameter (Fig. 3c).

Overall, both experiments resulted in 8 out of 17 of anestrous ewes having an ovarian and endocrine response to the GnRH challenge. Three of these ewes ovulated and formed a subsequent, normal CL; the other five ewes developed large luteinized follicular cysts. The failure to obtain a higher rate of ovulation could be attributed to an inadequate release of LH after GnRH stimulation but this does not appear to be the case. The LH profiles of all ewes in exp. 2 indicate that the individual response of ovulating, cystic or non-responding ewes was unrelated to the pattern of the LH surge elicited.

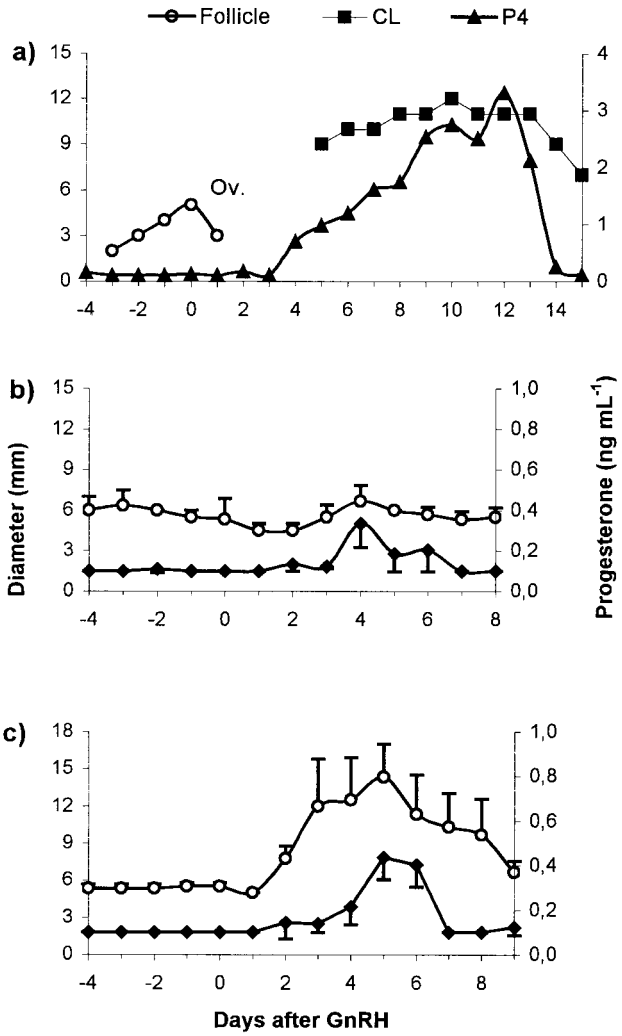

Fig. 3. Progesterone (P4) serum concentrations (mean ± SEM) and follicle and CL diameter profiles assessed by transrectal ultrasonography of ewes treated with a GnRH analogue during the seasonal anestrous (exp. 2): a) ewe that ovulated (Ov.) and underwent normal CL development; b) ewes without ovarian response; c) ewes that responded with formation of large, luteinized follicular cysts.

Furthermore, heights and intervals of the LH surge induced by the GnRH challenge were similar to those found normally during the ovine estrous cycle (Rawlings and Cook 1993). Hence, ovulatory response after GnRH treatment is less dependent on LH release than on the status of the follicles as was suggested in previous studies (McNeilly and Land 1979). In support of this concept, our ultrasonic study (exp. 2) shows that in the ovulating ewe the largest follicle was in the growing phase at time of GnRH injection. By contrast, in the other six ewes (non-ovulating) the large follicles at this time were in the static or regressing phases of development. An increase in intraovarian LH receptors is necessary for complete follicular development and ovulation. LH receptor numbers increase as follicles grow and decrease as follicles start to undergo atresia as a result of changes in the endocrine milieu (Roche 1996). Thus, inadequate concentrations of follicular LH receptors in "aged" follicles could explain the failure of these ewes to ovulate.

Unexpectedly, the ovarian response observed in this study differed from that described previously where a single injection of GnRH resulted in ovulation and the subsequent formation of short-lived CL (Hunter 1991). In our work, direct observation (exp. 1) and ultrasonic study (exp. 2) of the ovaries showed that cystic follicle formation was a common response. No short-lived CL [small, ≤ 4 mm in diameter at days 5 to 6; pale and non protruding from the ovarian surface (Rubianes et al. 1996b)] was observed at laparotomy (exp. 1). In exp. 2 the only ovulating ewe had a normal CL ultrasonic image and normal endocrine profiles. Nevertheless, the progesterone profiles of the five ewes developing cystic structures (total of both experiments) were similar to those reported for ewes with short-lived CL responses (Garverick et al. 1992), which was a transient (3–5 d) and small progesterone concentration increase (0.5 to 0.7 ng mL–1). Therefore, both ovarian responses (i.e. formation of a short lived-CL after ovulation and cyst formation after an ovulatory failure) had a similar endocrine pattern of a short luteal phase.

We concluded that without direct observation (i.e. laparoscopy or laparotomy) or, more appropriately, an ultrasonic day-to-day approach, the study of progesterone profiles alone could induce erroneous interpretations regarding ovarian response to a GnRH challenge. Further studies of the characteristics of the induced luteal structure assessed by an ultrasonic approach, particularly determining the relationship between the status of the largest follicle and its response to the LH surge, are needed to improve our knowledge about ovarian response to gonadotropins in anestrous ewes.

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