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Follicular recruitment and ovulatory response to FSH treatment initiated on Day 0 or Day 3 postovulation in goats

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Abstract

The present study evaluates the effect of the presence of a large growing follicle at the onset of superovulatory treatment on follicular recruitment and ovulatory response in dairy goats. The treatment consisted of six equal doses of pFSH given every 12 h (total dose: 200 mg NIH-FSH-P1) which was initiated at Day 0 (Group D0) or Day 3 (Group D3) postovulation. Two half-doses of an analogue of prostaglandin F2 α (delprostenate, 80 μ g each) were administered together with the last two FSH doses to ensure luteolysis. A dose of a GnRH analogue (busereline acetate, 10.5 μ g) was administered at the onset of estrus. Ovarian changes were evaluated twice a day by transrectal ultrasonography. Follicles were classified according to follicular diameter as small (3 to <4 mm), medium (4 to <5 mm) and large follicles (\geq 5 mm). The number of corpora lutea (CL) was recorded after laparotomy performed 6 days after estrus. The work was conducted in replicates. In the first trial, the does were assigned to either the D0 ($n = 4$) or D3 group ($n = 4$) and in the second replicate, each goat was assigned to the alternate group. No large follicles were recorded and the diameter of the largest follicle was 3.3 ± 0.1 mm (mean \pm S.E.M.) at the initiation of the treatment in D0-treated goats. In contrast, a growing large follicle was present (6.7 ± 0.4 mm, $P < 0.01$) when the treatment was initiated in D3-treated goats. In these goats, the number of small follicles increased 24 h after ovulation but then declined 48 h later, temporally correlated with the growth of the largest follicle of the first follicular wave. The number of small follicles recruited by the FSH treatment was significantly higher and occurred earlier in D0- than in D3-treated goats (9.0 ± 1.3 versus 5.6 ± 1.1 follicles; $P < 0.05$; and 24 h versus 48 h from the onset of the treatment, respectively). The number of large follicles at the onset of estrus was higher in D0- than in

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D3-treated goats (14.4 ± 1.9 versus 10.3 ± 1.3 ; $P < 0.05$). Consequently, the number of CL recorded 6 days after estrus were higher in D0- than in D3-treated goats (13.6 ± 1.9 versus 10.4 ± 1.9 ; $P < 0.05$, respectively). These results demonstrate that the presence of a dominant follicle at the time of initiation of super-stimulatory treatment is detrimental to ovulatory response. This study supports the advantages of the so-called Day 0 protocol, e.g. treatment starting soon after ovulation, when the emergence of the first follicular wave takes place and there are no dominant follicles.

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1. Introduction

The introduction of transrectal ultrasonography as a tool to study ovarian physiology provided information about follicular changes occurring during the estrous cycle and the wave-like pattern of follicular development in many species [1]. In each wave, a pool of small antral follicles emerges stimulated by a previous increase in FSH plasma concentrations. At least one of these follicles is selected and continues to grow while the other follicles undergo atresia. The selected (or dominant) follicle ovulates if progesterone levels decrease, which allows the development of positive feedback between the large follicle and the hypothalamus–pituitary axis that ends with ovulation.

The ovarian response obtained after stimulatory treatment in ruminants has been related to many factors [2,3]. It is known that the variability observed in response to superovulatory trials is associated with the presence or absence of a large growing follicle at the onset of treatment in cattle [4] and sheep [5]. The advantages of initiating superovulatory treatment at the time of emergence of a follicular wave (i.e. in the absence of a dominant follicle) have been demonstrated. Various commercial protocols have been designed in cattle using these findings [6]. In sheep, few studies have been conducted taking this concept into consideration, but successful results have been obtained using the so-called “Day 0 protocol” [7]. Treatment with FSH initiated soon after ovulation (Day 0) increased follicle recruitment, ovulation rate, embryo quality and the number of embryos recovered compared to treatment initiated 3 days after ovulation when an active growing follicle was present in the ovary [7]. Previous reports in goats [8–10] showed that follicular development also occurs in a wave-like pattern and it was proposed that at least during the first wave the phenomenon of follicular dominance is present [8]. Traditional protocols for ovarian superstimulation used commercially in goats consist of a long priming (11–18 days) with progesterone/progestagen devices and the administration of gonadotropin (FSH or eCG) previous to or around the time of the device withdrawal. These protocols do not take into account new information about ovarian follicular growth. Moreover, the effect on ovarian of the follicular status at the onset of the superstimulatory treatment response has not been yet studied in goats.

In the present work, we focused on the study of follicular dominance early in the ovulatory cycle in goats. We tested the hypothesis that the ovarian response is higher when the superstimulatory FSH treatment is initiated soon after ovulation, e.g. at the time of the emergence of the first follicular wave.

2. Materials and methods

2.1. Animals and treatments

The study was conducted during the breeding season (March–May) at the Laboratory of the Physiology of Reproduction of the Faculty of Veterinary Sciences (Montevideo, Uruguay, 35 °C SL). Eight multiparous nonlactating Alpine goats weighing 49.5 ± 3.8 kg (mean \pm S.E.M.) and with a mean body condition score of 2.9 ± 0.1 (scale: 0, emaciated; to 5, obese) were used. The daily food was alfalfa hay and pellets (mean: 1500 and 700 g per goat, respectively), and water was available ad libitum. The does were housed outdoors in a sheltered pen (30 m \times 30 m), and indoor box stalls (3 m \times 3 m) were used for ultrasonic examinations.

The estrous cycle was synchronized with the insertion of a controlled internal drug release device (CIDR-G, 0.3 g progesterone, Eazi Breed, InterAg, New Zealand), which was removed after 7 days. An i.m. dose of a prostaglandin F₂ α analogue (delprostenate 160 μ g, Glandinex, Universal Lab, Montevideo, Uruguay) was given at the time of CIDR insertion. Estrous behavior was detected twice a day without allowing the complete mount of the buck. Ovulation was detected as the collapse of a large follicle (≥ 5 mm) using transrectal ultrasonography. The superovulatory treatment consisted of six equal i.m. doses of FSH (Folltropin V, Vetrepharm, Ontario, Canada) given every 12 h (total dose equivalent to 200 mg NHI-FSH-P1). The goats received two i.m. half-doses (80 μ g) of the PGF₂ α analogue together with the last two FSH doses to ensure luteolysis. Estrous behavior was detected at 4-h intervals beginning at time of first PGF₂ α dose. An i.m. dose of a GnRH analogue (busereline acetate, 10.5 μ g, Receptal, Hoechst, Argentina) was given at the onset of estrus. The goats were allocated randomly into one of two groups (D0 and D3, four goats in each) and assigned to the experimental treatments. In Group D0, the FSH treatment was initiated when ovulation (Day 0) was determined by ultrasonography, and in Group D3, the treatment started 3 days after ovulation. The first dose of FSH was considered as Hour 0. The experiment was replicated 6 weeks later using the same goats but each goat was assigned to the alternate treatment.

2.2. Ultrasonography

Ovarian images were obtained by transrectal ultrasonography at 12-h intervals using a B-mode machine and 5.0 MHz linear array transducer (Aloka 210, Tokyo, Japan). A slightly arched plastic open tube (diameter, 2.5 cm; length, 40 cm) was fastened to the transducer with duct tape so that the probe could be manipulated externally into the rectum. The diameter and positions of antral follicles (≥ 3 mm) were recorded by a scan of both ovaries. The antral follicles were classified as small, medium and large follicles (3 to <4 mm, 4 to <5 mm and ≥ 5 mm in diameter, respectively). Examinations were also recorded on videotape, one tape per goat (Sony, Tokyo, Japan), for further analysis of data. The ultrasonography examinations were carried out from the beginning of the synchronized estrus until 3 days after the end of each of the superovulatory treatment periods. A laparotomy was performed 6 days after estrus to record the total number of CL present on the ovaries.

2.3. Hormone assays

Blood samples (10 ml) were collected daily by jugular venipuncture into vacutainer tubes for progesterone assay. Samples were centrifuged within 1 h of collection and serum was stored at -20°C until assayed. A direct solid-phase RIA (DPC, Diagnostic Product Co., Los Angeles, CA, USA) was used to determine the progesterone concentrations. The intra- and inter-assay coefficients of variation were 6 and 9%, respectively.

During the second replicate additional blood samples (2 ml) for LH determination were taken every 4 h from the onset of estrus (just before GnRH administration) until 28 h after. Luteinizing hormone concentrations were determined in duplicate using the procedure described by Forsberg et al. [11]. The minimum detectable level of LH was 0.2 ng/ml; all samples were determined in the same assay and the intra-assay coefficient of variation was 8%.

2.4. Statistical analyses

The diameter of the largest follicle when the superovulatory treatment was initiated and the number of CL at laparotomy were compared between groups by paired Student's *t*-test. The number of the different classes of follicles recorded every 12 h from the beginning of the FSH treatment until the onset of estrus, and the changes in progesterone concentrations were analyzed by the general linear model procedure of the Statistical Analysis System [12]. The statistical model included the effects of treatment group, time and the interaction between treatment group and time, and also the random effect of goat within treatment group. Data are presented as mean \pm S.E.M., and differences are considered to be significant when $P < 0.05$.

3. Results

3.1. Follicular status at the initiation of treatment

The mean intervals between CIDR withdrawal to estrus and ovulation preceding the superovulatory treatment for pooled groups were 39.8 ± 6.3 and 77.3 ± 6.3 h, respectively. The mean interval between the onset of estrus and ovulation was 38.3 ± 1.6 h.

Fig. 1 shows the growth profile of the largest follicle of the first follicular wave and the changes in the mean number of small follicles during the first 3 days after ovulation in Group D3 (e.g. before starting the FSH treatment). The largest follicle grew continually attaining a diameter of 6.7 ± 0.4 mm at 72 h after ovulation. The number of small follicles increased during the first day (0 h = 1.9 ± 0.7 follicles; 24 h = 6.0 ± 0.7 follicles, $P < 0.05$) but 1 day after, decreased significantly (48 h = 2.5 ± 0.6 follicles; $P < 0.05$).

In Group D0 the largest follicle present in the ovaries at the onset of superovulatory treatment was significantly smaller than in Group D3 (3.3 ± 0.1 mm versus 6.7 ± 0.4 mm; $P < 0.01$). At the time that treatment was initiated, the mean number of small follicles in Group D0 (4.0 ± 0.8 follicles) was greater than in Group D3 (1.6 ± 0.2 follicles; $P < 0.05$).

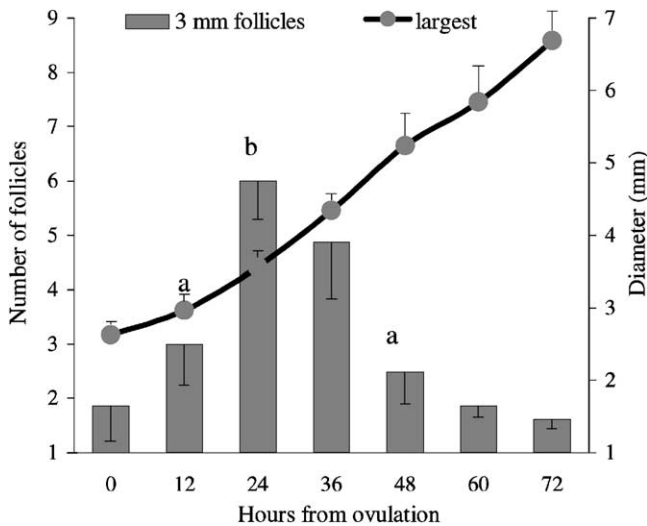


Fig. 1. Growth profile of the largest follicle of the first follicular wave and changes in the mean number (\pm S.E.M.) of small follicles (3 to <4 mm in diameter) during the first 3 days after ovulation for the eight D3-treated goats (v.gr. before FSH treatment) (a vs. b: $P < 0.05$).

3.2. Ovarian response to FSH treatment

Fig. 2 shows the changes in the mean number of small, medium and large follicles in both groups normalized to the onset of FSH treatment. The recruitment of small and medium follicles promoted by the FSH treatment was higher and occurred earlier in D0-treated goats. The maximum number of small follicles recruited in D0-treated goats (9.0 ± 1.3) was observed at 24 h from the initiation of the treatment, while the maximum number of small follicles recorded in D3-treated goats (5.6 ± 1.1 , $P < 0.05$) was observed 48 h from the onset of treatment. The maximum number of medium follicles recorded in D0-treated goats (7.5 ± 1.9 follicles at 36 h) was also higher and occurred earlier than in D3 goats (4.5 ± 0.8 follicles at 60 h, $P < 0.05$). At 48 h and thereafter, the number of large follicles was significantly higher in Group D0.

The intervals from the last FSH dose to the onset of estrus did not differ between groups (15.3 ± 3.3 h versus 17.0 ± 4.7 h, for D0- and D3-treated goats, respectively).

Table 1 shows the number of large follicles at the onset of estrus and the number of CL recorded directly at laparotomy performed 6 days after estrus.

3.3. Progesterone and LH assays

Mean serum progesterone concentration in D3-treated goats was 1.6 ± 0.4 ng/ml at the onset of FSH treatment and from then increased until the first PGF 2α dose (2.7 ± 0.9 ng/ml). The concentration of progesterone then decreased to 0.5 ± 0.2 ng/ml ($P < 0.05$). In D0-treated goats, mean concentration of progesterone was 0.5 ± 0.2 ng/ml at Day 2 when the first PGF 2α dose was given. After superovulation, the progesterone concentration

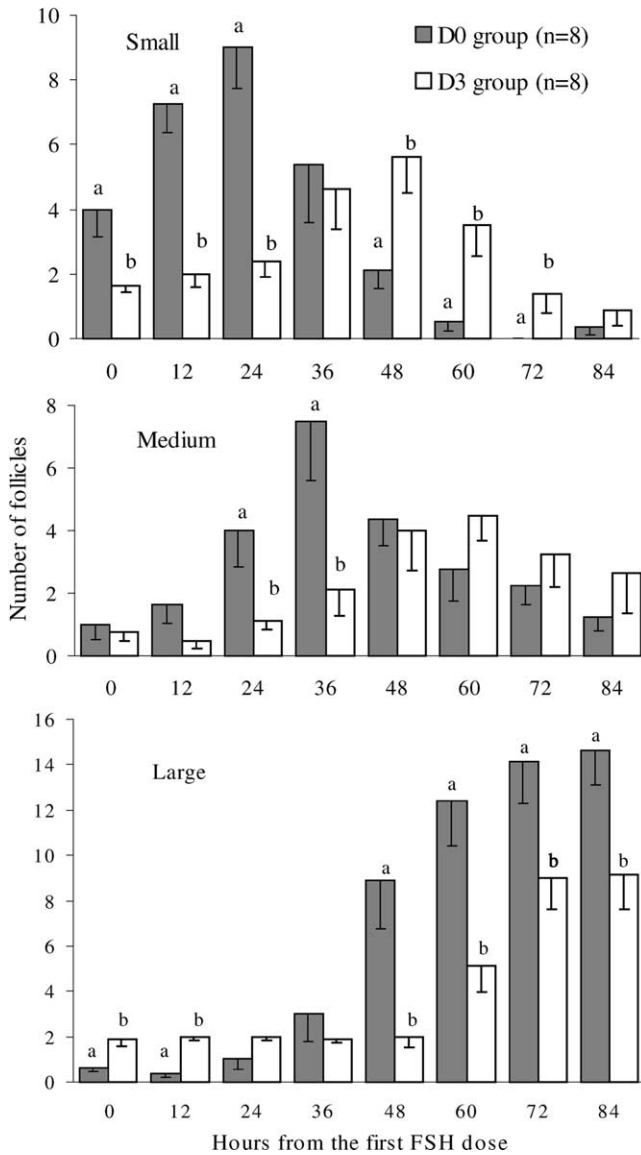


Fig. 2. Mean number (\pm S.E.M.) of small (3 to <4 mm), medium (4 to <5 mm) and large (\geq 5 mm) follicles normalized to the time of the first FSH dose in goats treated with a FSH superovulatory treatment initiated At the day of ovulation (D0 group) or 3 days later (D3 group) (for the same time and class, a vs. b: $P < 0.05$).

increased rapidly and similarly in both groups. In two D0-treated goats estrous behavior was observed again at the day of laparotomy, which was consistent with a decrease in progesterone concentration and a small and pale CL found in these animals.

The GnRH administration elicited an LH surge in the eight assayed goats. These values did not differ in D0 and D3 goats and were pooled. Basal LH concentrations just before

Table 1

Number (mean \pm S.E.M.) of large follicles (≥ 5 mm) at the end of FSH treatment, at the onset of estrus, and number of CL recorded by laparotomy performed 6 days after estrus in goats superstimulated with a FSH treatment initiated at Day 0 (D0 group, $n = 8$) or Day 3 (D3 group, $n = 8$) after ovulation

Group	End of FSH	Onset of estrus	Number of CL
D0-treated	14.1 \pm 1.9 ^a	14.4 \pm 1.9 ^a	13.6 \pm 1.9 ^a
D3-treated	9.0 \pm 1.4 ^b	10.3 \pm 1.3 ^b	10.4 \pm 1.9 ^b

In the same column, superscripts a and b differ significantly ($P < 0.05$).

GnRH administration averaged 0.7 ± 0.1 ng/ml whereas the maximum LH peak occurred 4 h after of GnRH dose and averaged 23.6 ± 3.1 ng/ml (range: 15.2–35.5). The duration of the induced LH surge lasted 8–12 h in all goats.

4. Discussion

The number of small follicles increased immediately after ovulation and before initiation of treatment in D3-treated goats, (e.g. emergence of the first wave). The largest follicle was actively growing more than 4 mm in size 2 days after ovulation; this was temporally correlated with a significant decrease in the population of small follicles. The inverse relation between the growth of the largest follicle and the total number of small follicles is one of the components of follicular dominance. Similar observations were described previously in goats supporting the theory that follicular dominance occurs during the first follicular wave [6,8]. This phenomenon has been extensively studied in cattle [13,14] and was described during the first wave and the ovulatory wave in ewes [7,15,16]. The present study confirms that follicular dominance occurs during the early luteal phase in goats.

At the onset of the FSH treatment the number of small follicles was higher in D0 goats. The FSH treatment promoted an increase in the population of small follicles (24 h), which agrees with the increase in the number of medium follicles found at 36 h, and is also consistent with the increase of large follicles at 84 h. The presence of a large follicle at the time of the onset of treatment delayed this follicular recruitment pattern (D3 goats). The total number of small and medium follicles started to increase only after the fourth and fifth FSH dose. This delay shows an inhibitory effect of the presence of a large (dominant) follicle on follicular recruitment. Results observed in the present work are similar to those observed using a similar experimental design in ewes [7]. Follicular dominance appears to be controlled by a number of mechanisms acting in concert [17]. These include alterations in endogenous FSH concentrations caused by estradiol and inhibin secreted by the dominant follicle during the growth phase, as well as the production of local ovarian factors, which can directly inhibit the development of subordinate follicles.

The ovulatory rate was also lower when a dominant follicle was present at the time treatment was started. This response could be the result of delay in follicular recruitment due to the presence of a large follicle, thus resulting in a lower number of large follicles at the onset of estrus and a lower ovulation rate. An interesting observation is that the number

of large follicles recorded by ultrasonography at time of the onset of estrus is correlated with the number of CL recorded at laparotomy. This relationship could be used to predict ovulatory success in a superovulatory program [18]. Overall, these results support the advantages of the use of superstimulatory protocols in goats initiated in the absence of a dominant follicle. One possibility is the use of the so-called “Day 0 protocol”, starting the FSH treatment immediately after ovulation is detected by ultrasonography or estimated 40 h after the onset of estrus.

In summary, the present work shows that the increase in the number of small follicles that occurs soon after ovulation (emergence of the first follicular wave) is suppressed correlatively with the growth of a large follicle, confirming that follicular dominance is present during the early luteal phase in the goat. The dominant effect is also evident when a superovulatory treatment is initiated in the presence of a large growing follicle. A delay and a decrease in follicular recruitment and a lower ovulation rate were also observed, supporting the advantages of initiating stimulatory treatment in the absence of a large follicle in goats.

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References

- [1] Adams G. Comparative patterns of follicle development and selection in ruminants. *J Reprod Fertil* 1999;54(Suppl):17–32.
- [2] Cognie Y. State of the art in sheep–goat embryo transfer. *Theriogenology* 1999;51:105–16.
- [3] Monniaux D, Chupin D, Saumande J. Superovulatory response in cattle. *Theriogenology* 1983;19:55–81.
- [4] Guilbaut LA, Grasso F, Lussier JG, Roullier P, Matton P. Decreased superovulatory responses in heifers superovulated in the presence of a dominant follicle. *J Reprod Fertil* 1991;91:81–9.
- [5] Rubianes E, Ibarra D, Ungerfeld R, Carbajal B, de Castro T. Superovulatory response in anestrous ewes is affected by the presence of a large follicle. *Theriogenology* 1995;43:465–72.
- [6] Bo GA, Adams GP, Pierson RA, Mapletoft RJ. Exogenous control of follicular wave emergence in cattle. *Theriogenology* 1995;43:31–40.
- [7] Rubianes E, Ungerfeld R, Viñoles C, Rivero A, Adams GP. Ovarian response to gonadotropin treatment initiated relative to wave emergence in ultrasonographically monitored ewes. *Theriogenology* 1997;47:1479–88.
- [8] de Castro T, Rubianes E, Menchaca A, Rivero A. Ovarian dynamics, serum estradiol and progesterone concentrations during the interovulatory interval in goats. *Theriogenology* 1999;52:399–411.
- [9] Ginther OJ, Kot K. Follicular dynamics during the ovulatory season in goats. *Theriogenology* 1994;42:987–1001.
- [10] Gonzalez de Bulnes A, Santiago Moreno J, Gomez-Brunet A, Inskip EK, Townsend EC, Lopez-Sebastian A. Follicular dynamics during the estrous cycle in dairy goats. *Anim Sci* 1999;68:547–54.
- [11] Forsberg M, Tagle R, Madej A, Molina JR, Carlsson MA. Radioimmunoassay of bovine, ovine and porcine luteinizing hormone with a monoclonal antibody and a human tracer. *Acta Vet Scand* 1993;34:255–62.

- [12] Statistical Analysis Systems Institute Inc., 1989. SAS User's Guide: Statistics. SAS Institute Inc., Cary, NC.
- [13] Ginther OJ, Wiltbank MC, Fricke PM, Gibbons JR, Kot K. Selection of the dominant follicle in cattle. *Biol Reprod* 1996;55:1187–94.
- [14] Ginther OJ, Bergfelt DR, Kulick LJ, Kot K. Selection of the dominant follicle in cattle: role of estradiol. *Biol Reprod* 2000;63:383–9.
- [15] Bartlewsky PM, Beard AP, Cook SJ, Chandolia RK, Honoramooz A, Rawlings NC. Ovarian antral follicular dynamics and their relationships with endocrine variables throughout the estrus cycle in breeds of sheep differing in prolificacy. *J Reprod Fertil* 1999;115:111–24.
- [16] Ginther OJ, Kot K, Wiltbank MC. Associations between emergence of follicular waves and fluctuations in FSH concentrations during the estrus cycle in ewes. *Theriogenology* 1995;43:689–703.
- [17] Armstrong DG, Webb R. Ovarian follicular dominance: the role of intraovarian growth factors and novel proteins. *Rev Reprod* 1997;2:139–46.
- [18] Menchaca A, Pinczak A, Rubianes E. Ultrasonographic estimation of the ovulation rate and the length of the ovulation period in superovulated goats. *Theriogenology* 2001;55:531 (abstract).