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A simple method to select high superovulatory responder goats

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ABSTRACT

In most female mammals, a common drawback to multiple ovulation embryo transfer programs is the variability in the superovulatory response to the multidose pFSH treatment. The aim of the present study was to identify embryo donor goats based on their response to superovulation before the performance of a high-cost hormonal treatment, as we have previously done in sheep. To this end, we evaluated the number of ovulations obtained in response to the administration of a one-shot eCG treatment and related it with the subsequent ovarian response to a multiple-dose pFSH treatment in 33 goats of the Criolla-Neuquina breed. Goats received a one-shot eCG treatment of 800 IU at the end of a 17-day progestational treatment; 9 days later, started a second 17-day progestational treatment and then received a multiple-dose pFSH treatment on days 15–17 (116 mg pFSH, in six decreasing doses). The number of corpora lutea (CL) per goat was recorded laparoscopically after both hormonal treatments. On day 8 after the second pessary removal, embryos were surgically recovered and classified by quality. Results showed a significant positive correlation between the number of CL obtained in response to the eCG and pFSH treatments ($r = 0.41$; $y = 0.8352x + 6.9906$; $P < 0.05$), although of limited value to select high ovulatory responding goats, due to its low correlation value. Then, goats were grouped into high and low ovulatory responders to the eCG (High ≥9; Low <9 CL) and pFSH treatments (High ≥13; Low <13 CL). After the eCG and pFSH treatments, 60% of the goats maintained their classification as high or low superovulatory responders (expressed as recurrence rate). Significant differences were found in the number of CL (18.2 ± 1.3 vs 9.9 ± 1.3), number of embryos + oocytes (13.5 ± 1.7 vs 7.6 ± 1.7), number of embryos (10.8 ± 1.4 vs 5.1 ± 1.4) and number of Grade 1 and Grade 2 embryos (8.8 ± 1.4 vs 4.3 ± 1.4) between high and low superovulatory responder goats ($P < 0.05$), while no differences were observed in the number of oocytes and in the recovery of embryos + oocytes, embryos, Grade 1 and Grade 2 embryos and fertilization rates ($P > 0.05$). In conclusion, the recurrence rate in ovarian response between the one-shot eCG treatment and the multiple-dose pFSH treatment would confirm the existence of an "individual or intrinsic factor" of the donor goat that would respond as a high or low ovulatory responder to superovulatory treatments.

1. Introduction

The use of multiple ovulation and embryo transfer (MOET) technology has made substantial contributions to the genetic improvement of small ruminants in several countries around the world. However, this technology has been slowly accepted, mainly due to the variability of the superovulatory response, which leads to higher costs of embryo production. The main factors that influence the variability in the response to superovulation (SOV) treatments are the type of hormonal treatment, the breeding season, the follicular state of the ovary, and the genetics and nutritional status of the animals. These factors may be involved directly or indirectly

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in the program results, by affecting the quality of the oocyte, the embryo, and/or the synchronization of multiple ovulations [1]. A determinant factor affecting the success of embryo transfer is the unpredictable individual response to SOV [2].

The goal of superovulatory treatments is the production of a large number of viable embryos capable of establishing and maintaining pregnancy after embryo transfer. However, the determining factor for variable results is the high inherent variability in the number of corpora lutea (CL) and embryos obtained in response to SOV between individual animals of the same breed and treated group regardless of the species involved [3]. This individual effect is attributed to a high variability in the intrinsic number of ovarian follicles in different females [4], being, however, a repeatable character in the same animal [5,6]. The reasons for this variability between individuals are still unknown but can be attributed to intrinsic factors such as the number of primordial follicles at birth, genetic differences in the mechanisms involved and/or different hormonal levels [7]. Although discrepancies remain, the scarce or non-significant relationship previously found in sheep between populations of follicles of different diameter before the first dose of pFSH and the subsequent ovarian response reinforces the hypothesis that each donor female has a strong individual intrinsic factor that determines its own degree of response to SOV treatments [6]. These previous studies in sheep reported a significant repeatability in the magnitude of ovarian response to successive SOV treatments in the same ewe, evidenced by the reiterated recovery of a high number of transferable embryos in high responsive ewes [5,6].

Based on these results, we considered the feasibility of establishing a simple and low-cost methodology to select goats with high multiovulatory response prior to pFSH SOV treatments. Thus, the aim of the present study was to identify embryo donor goats based on their high intrinsic response to superovulatory treatments, obtained in response to the administration of a one-shot eCG treatment and the subsequent recurrent superovulatory response to a multiple-dose pFSH treatment. The development of a test to identify high responsive females with higher embryo production would avoid treatments and surgery procedures in low responsive females, avoiding unnecessary expenses and regarding ethical reasons.

2. Materials and methods

The study was conducted at the Laboratory of Reproduction in Small Ruminants, Experimental Station of the Instituto Nacional de Tecnología Agropecuaria, INTA, Bariloche, Argentina (latitude 41° 7′ S, longitude 71° 15′ W). All the experimental procedures with animals were carried out in accordance with national and international standards for the use and care of experimental animals [CICUAE INTA – PATNOR- Res. 533/16 Disp. CRPN 66/17; Protocol 02/2022].

A total of 33 4-year-old multiparous female goats of the Criolla-Neuquina breed, with good body condition (>3 out of 5; [8]) and 39.4 ± 1.9 kg body weight, were used during the breeding season. Animals were kept outdoors in a sheltered pen and received a live-weight maintenance nutritional ration throughout the experiment.

2.1. Selection test (1st protocol)

The predictive value of the selection test was determined by comparing the number of ovulations in response to the eCG treatment and the number of ovulations in response to the multiple-dose FSH treatment. The first protocol consisted of a 17-day progestational treatment (60 mg MAP, Progespon®, Syntex, Argentina) and a single dose of 800 IU of eCG (Novormon®, Syntex, Argentina) at the time of pessary removal (Fig. 1). Four days after the end of the eCG treatment, the number of CL was counted by laparoscopic observation, and PGF2α (125 μg im; Estrumate®, Schering-Plow, Argentina) was injected.

Laparoscopic observations were performed to determine the number of CL in response to SOV treatments. To this end, females were deprived of food for 24 h and of water for 12 h, and immobilized on a standard cradle for laparoscopic procedures under local anesthesia (2 mL, sc, lidocaine hydrochloride, Lidocaína 2%®; Over, Argentina). The surgical field, cranial to the udder, was shaved and disinfected and, then, a 4-mm endoscope (Richard Wolf, Knittlingen, Germany) was inserted into the abdominal cavity through a trocar, approximately 10 cm cranial to the udder and 5 cm to the left side of the midline, to visualize the ovaries. A Veress needle (Endopath®, Ethicon Endo-surgery, USA), placed in the midline, allowed the support of the ovaries from the utero-ovarian ligament, to present the ovarian surface for laparoscopic visualization. All laparoscopies were performed by the same and trained operator. Finally, the trocar orifices were treated with a local antibiotic-cicatrizing solution (Gentamicine, Genmicin®, Over, Argentina). Immediately after laparoscopic observation, a 125-μg dose of Cloprostenol (PGF2α; Estrumate®, Schering-Plow, Argentina) was applied to induce CL regression.

2.2. FSH superovulation treatment and embryo recovery (2nd protocol)

On day 5 after PGF2α administration, donor females received a second ovarian stimulation treatment consisting in the insertion of 17-day progestational pessaries (60 mg MAP, Progespon®, Syntex, Argentina) and the im administration of a multiple dose of 116 mg of pFSH (Folltropin®-V, Bioniche, Canada). The superovulatory treatment was applied on days 15–17 of the progestational treatment, in six decreasing doses every 12 h (28 mg x 2, 18 mg x 2 and 12 mg x 2). In conjunction with the fifth application of pFSH and the removal of the intravaginal sponge, 100 IU of eCG (Novormon®, Syntex, Argentina) were applied (Fig. 1). Estrus detection, intrauterine artificial insemination and embryo recovery were performed according to the procedures described by Gibbons et al. [9]. The onset of estrus was detected with the aid of an adult teaser buck (24–36 h after pessary removal), and donors were inseminated using laparoscopy, with frozen/thawed semen (2×10^8 sperm per doe), 12–14 h after the onset of the induced estrus.

On day 8 after pessary removal, the number of CL was recorded by laparoscopy as described in Section 2.1. Immediately after, embryos were surgically recovered under general anesthesia. Females, deprived of food for 24 h and of water for 12 h, were anesthetized with xylazine (4 mg, im, Kensol®, König, Uruguay) and ketamine (12.5 mg, im, Ketonal®, Richmond, Argentina). Local anesthesia was applied in the surgical field (2 mL, sc, lidocaine hydrochloride, Lidocaina® 2%, Over, Argentina). All embryos were collected by laparotomy, performed by the same operator, flushing each uterine horn with 20 mL of commercial embryo recovery medium (PBS; Dipla flash plus®, Argentina), pre-warmed to 38 °C and supplemented with 10% adult bovine serum (BAL®, Internegocios, Argentina). The embryo recovery medium was injected by means of a sterile syringe with an 18 G blunt needle, inserted close to the uterus bifurcation, and directed from the uterine horn towards the utero-tubal junction, where a catheter was attached with silk. Once both uterine horns were flushed, surgical incisions were closed by suture. General antibiotic was administered as penicillin-streptomycin (10,000 IU for each kg of weight, im, Estreptopendiben®, Biogenesis Bagó, Argentina), and local antibiotic-cicatrizing solution (Gentamicine, Genmicin®, Over, Argentina) was applied at the site of the abdominal incision. The recovered medium was emptied into sterilized Petri dishes. Embryos were recovered and

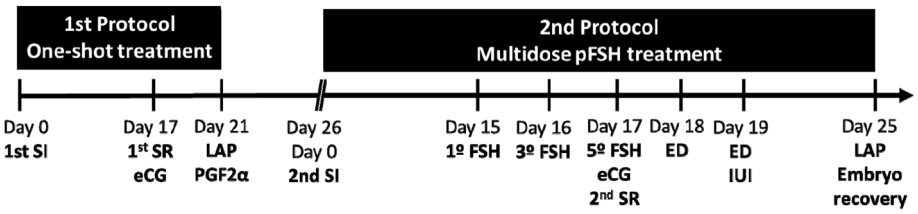

Fig. 1. Schedule of the selection test to identify high ovulatory responsive goats to superovulation treatments. SI: Sponge insertion, SR: Sponge removal, LAP: Corpora lutea counting by laparoscopy, ED: Estrus detection, IUI: Intrauterine Insemination.

examined under a stereomicroscope (Olympus SZ, Olympus Optical Co., LTA, Japan), and classified according to morphological criteria and using the guidelines of the International Embryo Transfer Society [10].

2.3. Indexes of superovulatory response

The number of goats exhibiting estrus in response to the eCG and pFSH treatments and the number of goats exhibiting premature CL regression in response to the pFSH treatment were recorded.

The following information was recorded for each goat: number of CL, number of embryos + oocytes recovered, number of embryos recovered, and number of Grade 1 and Grade 2 embryos recovered. The total rate of recovery was obtained, for each animal, by dividing the number of embryos + oocytes recovered by the number of CL. The embryo recovery rate was calculated as the number of embryos recovered by the number of CL. The rate of recovery of embryos of Grade 1 and Grade 2 was obtained by dividing the number of recovered Grade 1 and Grade 2 embryos by the number of embryos recovered. The fertilization rate was expressed as the quotient between the number of embryos and the number of embryos + oocytes recovered. All rates are expressed as percentages.

To evaluate the one-shot eCG treatment as a tool for donor selection, the recurrence rate, defined as the ovarian response after pFSH administration in relation to the previous response (high or low) after eCG administration, was calculated as previously [11]. To this end, goats were grouped into high and low ovulatory responders to eCG treatment (High $\geq$9; Low <9 CL) and pFSH treatment (High $\geq$13; Low <13 CL). The value of CL for the cutoff between groups of high and low ovulatory response was the one that left the same number of goats in each of the groups in response to the pFSH treatment (median value).

2.4. Statistical analysis

The data were analyzed using the R Commander package [12]. Simple linear regression analysis was performed to assess the relationship between the number of CL obtained in response to the one-shot eCG treatment and the multidose pFSH treatment. Analysis of variance (ANOVA) was used to compare embryo production and rates of recovery and fertilization between low and high ovulatory responder goats to pFSH treatment. Statistical treatment of results expressed as percentages was performed after arcsine transformation of the values for each individual percentage. Results are expressed as mean ± SEM and a value of $P < 0.05$ was considered statistically significant.

3. Results

All goats exhibited estrus in response to the eCG treatment, while 8 out of the 33 did not show estrus in response to the pFSH treatment. Two out of these 25 does exhibited premature CL regression in response to the pFSH treatment, from which only oocytes were recovered.

A mean value of 8.7 ± 0.5 CL per donor was observed in response to the one-shot eCG treatment, and an increase in the mean number of CL was observed in response to the multidose pFSH treatment, totalizing 14.2 ± 1.0 CL per donor goat.

When considering the ovarian response to both superovulatory treatments, a simple linear regression was found between the number of CL in response to the eCG treatment and the number of CL in response to the pFSH treatment ($r = 0.41$; $y = 0.8352x + 6.9906$; $P < 0.05$) (Fig. 2).

When donor goats were grouped as high and low ovulatory responders to eCG, the mean number of CL was 10.4 ± 0.5 CL in the high responders (CL $\geq$ 9; n = 15) and 6.1 ± 0.7 in the low responders (CL < 9; n = 10). When goats were grouped according to their response to the pFSH treatment, these values were of 18.2 ± 1.3 CL for the high responders (CL $\geq$ 13; n = 13) and 9.9 ± 1.3 for the low responders (CL < 13; n = 12).

Nine out of the 15 goats with high ovulatory response to eCG treatment showed a high ovulatory response to pFSH treatment (60%; 9/15), while 6 out of the 10 goats that presented a low ovulatory response to eCG treatment showed a low ovulatory response after pFSH treatment (60%; 6/10). These results indicate a rate of recurrence of 60% for high responder goats and of the same magnitude for low responder goats.

The ovarian response and embryo production in donor goats with high and low ovulatory response to pFSH treatment are shown in Table 1. Significant differences were found in the ovarian response, number of embryos + oocytes, number of embryos and

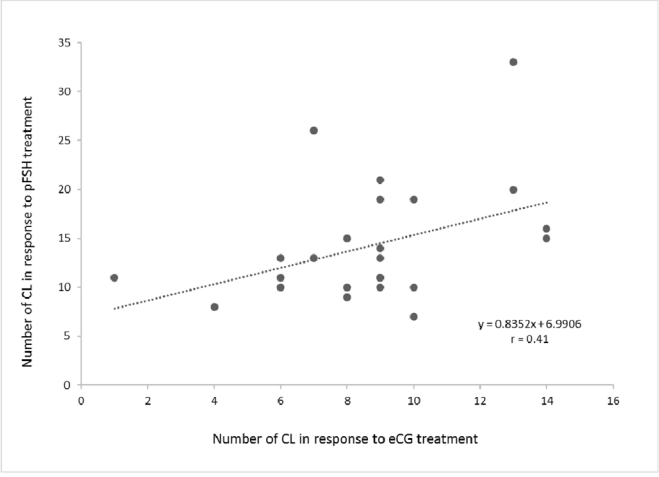

Fig. 2. Relationship between the number of corpora lutea (CL) in response to a treatment with 800 IU im of eCG (Novormon®, Syntex, Argentina) and the subsequent superovulatory response with the im application of 116 mg of pFSH (Folltropin ®-V, Bioniche, Canada) in goats.

Table 1
Ovarian response and embryo production in high and low ovulatory responder goats (High ≥ 13 corpora lutea; Low < 13 corpora lutea) grouped according to their response to a multidose pFSH treatment.

Embryo recovery	High (n = 13)	Low (n = 12)
Number of CL (± SEM)	18.2 ± 1.3[a]	9.9 ± 1.3[b]
Embryos + oocytes (± SEM)	13.5 ± 1.7[a]	7.6 ± 1.7[b]
Embryos (± SEM)	10.8 ± 1.4[a]	5.1 ± 1.4[b]
Oocytes (±SEM)	2.7 ± 1.2[a]	2.5 ± 1.2[a]
Grade 1 and Grade 2 embryos (± SEM) [a]	8.8 ± 1.4[a]	4.3 ± 1.4[b]
Total embryo + oocyte recovery (%)	73.5 ± 8.1[a]	74.8 ± 8.1[a]
Total embryo recovery (%)	61.5 ± 9.6[a]	51.0 ± 9.6[a]
Grade 1 and Grade 2 embryo recovery (%)	80.5 ± 10.4[a]	65.7 ± 10.4[a]
Fertilization rate (%)	83.1 ± 9.0[a]	71.9 ± 9.0[a]

[a, b] Different letters within columns indicate significant differences ($P < 0.05$).
[a] Grade 1 and Grade 2 [10].

number of Grade 1 and Grade 2 embryos between high and low responders to the pFSH treatment ($P < 0.05$), while no differences were observed between groups in the recovery rates of embryos + oocytes, embryos, Grade 1 and Grade 2 embryos and fertilization rate ($P > 0.05$).

4. Discussion

The present study evaluated the possibility of using a screening protocol to identify high and low ovulatory responder goats to FSH treatment. The 60% recurrence rate found suggests the need to find possible ways to improve the discrimination capacity of the eCG predictive test. Besides, to our knowledge, this is the first report quantifying differences in embryo yields between high and low ovulatory responders to superovulatory treatments in goats.

The aim of this study was to test the feasibility of establishing a practical criterion to identify donor goats of high ovulatory response to SOV treatments, reducing economic costs and avoiding stress and surgery procedures in poorly responding animals. The results showed a positive significant relationship between the number of CL obtained in response to the eCG and pFSH superovulatory treatments. However, the limited value of the correlation explaining less than 20% of the variability in the ovarian response to pFSH should be highlighted. To our knowledge, despite its limited value, this is the first study reporting a linear function between the ovulation response to both eCG and FSH treatments, unlike that found by Balaro et al. [13], who obtained a quartic regression to explain this relationship. The importance of enhancing this predictive classification test is highlighted by the fact that, despite improvements in both animal handling and gonadotropin administration protocols, high variability in ovarian response among animals cannot be avoided [14]. This could be improved by adding further information to the CL assessment. In this sense, some researchers seem to support the view that ultrasound screening of the mean value of follicles during the first follicular wave would allow selecting females with greater responding capacity to SOV treatments in ewes [7]. Nevertheless, this discrimination test has not yet been tested in goats.

The cut-off point chosen as the median of the distribution allowed identifying high and low ovulatory responder goats, due to a consistent recurrence rate. The eCG pre-selection test had been previously proven in sheep and allowed the identification of high-responding and low-responding ewes under different experimental conditions [3,11]. However, in some studies, such as that by Pinto et al. [15], the authors were not able to classify donors according to their ovarian response to eCG, after animals underwent a short-wave synchronization protocol and subsequent SOV treatment. In goats, the only recurrence study so far reported [13] positively discriminated between high and low responder donors by applying a one-shot eCG treatment, prior to the application of a multiple pFSH treatment, although the authors did not quantify embryo production. Brasil [3] evaluated different alternatives to increase *in vivo* embryo production and reduce the variability in SOV treatments and concluded that SOV yields in ewes can be improved by selecting donors with a history of high superovulatory response.

As mentioned above, unlike our present results in goats, in our previous study in sheep, we demonstrated a high linear positive correlation between the number of CL in response to the eCG and FSH treatments [11]. Possible explanations for this difference require further study, but may be related to the low CL variability found in response to SOV treatments in the current study when compared to the higher CL variability found in the previous study. Also, Balaro et al. [13] postulated that, in the mentioned study [11], sheep showed a lower number of large follicles than that observed in their study in goats, encouraging fact to consider different effects of goat and sheep follicle populations on superovulatory responses.

When considering animal welfare, emphasis should be made in the application of non-surgical procedures to classify goat donors as low or high responders. In the present study, the laparoscopic technique was applied to assess the exact number of CL, which would not have been possible by ultrasonography due to the superposition of the CL in the image when the response is very high. In this regard, goats could be screened for possible poor responders after the one-shot eCG treatment by a non-invasive ultrasonography method, thus avoiding laparoscopic procedure.

Several physical and physiological criteria can be assessed to aid in predicting the superovulatory outcome in sheep and goats, which in turn would allow selecting the best SOV responders. These criteria include the measurement of antral follicular blood flow [16,17], inhibin A concentration [18,19], estradiol concentration [20,21], progesterone concentration during diestrus [22], rate of recurrence between eCG and pFSH treatments [11,13], levels of anti-Müllerian hormone (AMH) [15,23,24] and characterization of the follicular status [6,13,14,18]. By using ultrasound as a means of assessing the ovarian follicle population, Mossa et al. [7] concluded that ewes with a higher number of follicles ≥8 mm in diameter at the beginning of the estrous cycle had a better superovulatory response in terms of more CL and higher quality of the embryos recovered.

Hormonal determinations or sonographic assessment of specific antral follicular blood flow velocities can provide a useful non-invasive method to predict the outcome of the superovulatory treatment in ewes [17]. The subsequent control of these potential endocrine predictors promises to aid in the development of superovulatory treatments that produce optimal and predictable results. However, hormonal determinations and color Doppler sonography have a high cost, being economically unaffordable for field veterinarians who work in the reproduction of small ruminants. Our proposal of the eCG predictive test only requires having an ordinary ultrasound equipment, which, in turn, has other uses among professionals in their daily practice, such as pregnancy diagnosis in field conditions. In our study, when donor goats were selected as high or low ovulatory responders to pFSH treatment, data evidenced that the number of CL in high responders (18.2) almost doubled values in low responder goats (9.9). The same was observed for the number of total embryos (10.8 and 5.1 for high and low responders, respectively) and transferable (Grade 1 and Grade 2) embryos (8.8 and 4.3 for high and low responders, respectively), confirming the importance of the method here proposed for the selection of donors in accordance to their eCG-SOV response prior to the high-cost pFSH treatments. Therefore, the selection of donor goats through the recurrence between treatments with eCG and pFSH would seem to be a good predictive method to avoid the high

costs of hormonal determinations or color Doppler sonography.

5. Conclusion

The present results allow concluding that the recurrence rate in ovarian response between the one-shot eCG treatment and the multiple-dose pFSH treatment would confirm the existence of an "individual or intrinsic factor" of the donor goat that would respond as a high or low ovulatory responder to superovulatory treatments. The 60% recurrence rate found in this study suggests the need to find possible ways to improve the discrimination capacity of the eCG predictive test, allowing its implementation as a way to select best responding females prior to their admission into a MOET program. The availability of this technology would allow reducing costs and avoiding unnecessary treatments and surgeries by preventing the use of animals with low response in superovulatory and embryo transfer programs.

CRediT authorship contribution statement

María Macarena Bruno-Galarraga: Methodology, Investigation, Data curation, Writing – original draft, Project administration. **Jimena Fernandez:** Investigation, Resources, Data curation. **Isabel María Lacau-Mengido:** Writing – review & editing. **Antonio Gonzalez-Bulnes:** Writing – review & editing. **Alejandro Gibbons:** Conceptualization, Methodology, Funding acquisition. **Marcela Cueto:** Conceptualization, Validation, Formal analysis, Writing – review & editing, C, Supervision.

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