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Pre-Selection Test to Identify High Responder Donor Goats

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Contents
The aim of the study was to evaluate the feasibility of pre-selection of high or low responder does prior to the superovulatory protocols. Twenty Saanen does received 800 IU of equine chorionic gonadotropin (eCG) at the end of long-term progestogen treatment. Fourteen days later, a second progestogen protocol associated with a multiple-dose follicle stimulation hormone (FSH) treatment (5 IU/kg of FSH, in six decreasing doses between days 4 to 6 of the protocol) was administered. Transrectal ultrasound was used to assess the follicular status at the beginning of superovulatory treatments, at the oestrous onset and on the seventh day of the oestrous cycle for counting corpora lutea (CL). A significant lower number of CL was obtained in eCG-treated in comparision with FSH-treated does ($p < 0.05$). A quartic regression was able to explain the relationship between the number of CL in response to both treatments (r^2=0.50; $p < 0.05$). Seventy per cent (14 of 20) of does maintained the same ovulatory response (high or low) after treatments. The Kappa ($\kappa = 0.40$; $p < 0.05$) and Spearman (rs = 0.39; $p = 0.08$) coefficients were able to show a relationship between treatments. Regarding the follicular status, there is a significant relationship between the number of small follicles ($r = 0.71$; r^2=0.47; $p < 0.01$) and total follicles ($r = 0.60$; $p < 0.01$) at eCG and first FSH dose with the number of CL. Moreover, it was found a negative relationship between the presence of large follicles and the number of CL in response to eCG treatment ($r = -0.44$; $p < 0.05$), but not from FSH ($p > 0.05$). In conclusion, the screening test with eCG has the potential to identify Saanen does that will better respond to the superovulatory protocol with FSH. In addition, it highlighted the importance of an ultrasound evaluation prior to the beginning of superovulatory treatments with FSH to characterize the follicular status and identify the potential donors of high ovulatory response in MOET programmes in goats.

Introduction

Goats are significant contributors to the global production of food and fibre. With the increase in world population, goats have an essential role, especially for the economy of developing countries (Pollot and Wilson 2009). To optimize breeding systems and commercial gains, the multiple ovulation and embryo transfer (MOET) programme appears with the potential to improve the number of offspring produced by genetically valuable goats (Baldassarre and Karatzas 2004).

The success of a MOET programme is determined by intrinsic (breed, age, nutritional and reproductive status) and extrinsic (source and purity of hormones and protocols of administration) factors (González-bulnes et al. 2004). Although most of these factors could be controlled, the MOET efficiency remains a challenge, mainly due to the high individual variability in the ovulation rate. Thus, the implementation of MOET programmes under field conditions has been limited for economic reasons related to its low reproductive efficiency (Amiridis and Cseh 2012).

In the last decade, researchers have been dedicated to elucidate the role of the ovarian follicular status at the beginning of superovulatory treatment (Menchaca et al. 2002, 2007, 2009) and new protocols have been employed to reach the ideal ovarian status at this time. Nevertheless, the individual variability remains a challenge. It has been proposed the existence of a high intra-individual repeatability in response to successive superovulatory treatments in sheep (Bruno-Galarraga et al. 2014), which could be related to the pool of primordial follicles, intrinsic to each ewe (Torres-Rovira et al. 2014). Thus, the identification of does as high or low responders to superovulatory treatments would be an interesting tool for MOET programmes.

Over the last years, few alternatives have been evaluated such as the description of screening hormonal protocols (Davis and Johnstone 1985; Lahoz et al. 2011), as well as Anti-Mullerian hormone quantification to identify potentially good embryo donors (Monniaux et al. 2011; Torres-Rovira et al. 2014). In sheep, pre-selection tests using a low dose of follicle stimulation hormone (FSH) or equine chorionic gonadotropin (eCG) have shown promising results in selecting pre-pubertal and adult ewes with good potential for embryo donors (Davis and Johnstone 1985; Lahoz et al. 2011; Torres-Rovira et al. 2014). Recently, it was demonstrated a significant satisfactory correlation (r^2= 0.63) between the superovulatory responses to the use of a first eCG treatment and a subsequent FSH treatment in sheep donors (Bruno-Galarraga et al. 2015). Therefore, the use of a screening protocol with only eCG, which is much cheaper than FSH, may be helpful to identify the best donors and to eliminate the so-called 'individual effect'. However, the reliability of the use of such screening test in goats is yet to be determined.

Thus, the aim of this study was to evaluate the feasibility of establishing a pre-selection test by the use of low dose of eCG to identify does as high or low responders, prior to the use of a multiple ovulation FSH

treatment. Concomitantly, the effect of follicular status at the beginning of eCG and FSH superovulatory treatments and their relationship with the number of corpora lutea obtained was assessed.

Materials and Methods

Experimental conditions and animals

The study was carried out at Santa Clara Farm, located in Coronel Pacheco (Minas Gerais, Brazil) at 21°S latitude under tropical conditions. This research was reviewed and approved by the Animal Care Committee of Universidade Federal Fluminense (number 374) and was conducted under the ethical principles of the Brazilian Society of Laboratory Animal Science.

Twenty healthy pluriparous Saanen does (4.7 ± 1.6 years old; 2.9 ± 0.3 of body condition score) were used during the non-breeding season (November and December). Animals were kept under intensive management and received 1.300 g/goat/day of concentrate (homemade mixture with 18% of crude protein), alfalfa and ryegrass hay and chopped elephant grass. Water and mineralized salt (Caprinofós®, Tortuga, São Paulo, Brazil) were provided *ad libitum*.

eCG superovulatory treatment – pre-selection test procedure

This test consisted of the insertion of a progestogen-impregnated intravaginal pessary (60 mg of medroxyprogesterone acetate, Progespon®, Schering Plough, São Paulo, Brazil) for 14 days, with pessary exchanged at 7th day. A dose of 800 IU IM of eCG (Novormon®, MSD Saúde Animal, São Paulo, Brazil) at time of pessary removal was administered. In sequence, the onset and duration of oestrous behaviour was detected twice daily (7 a.m. and 7 p.m.) from 24 h after pessary removal by adult fertile bucks.

FSH superovulatory treatment – Traditional multiple ovulation procedure

Fourteen days after eCG administration, a second progestogen-impregnated intravaginal pessary was inserted for 6 days. The superovulatory treatment was described previously by Fonseca (2006). Briefly, a total of 5 IU of FSH IM (Pluset®, Hertape Calier, Juatuba, Minas Gerais, Brazil) per kg of body weight was diluted into 20 ml of saline and administered in six decreasing doses (5-5-3-3-2-2 ml) twice daily from the afternoon of day 4 to day 7, 12 h after pessary removal. Oestrous behaviour was detected twice daily (7 a.m. and 7 p.m.) from 24 h after pessary removal with adult fertile bucks. Fifty-four hours after the end of the oestrus, all does received 500 IU IM of hCG (Vetecor®, Hertape Calier, Juatuba, Minas Gerais, Brazil) to control premature regression of CL.

Ultrasonographic evaluation

Transrectal ultrasound (Mindray Color–M5 VET™, Mindray Medical International Limited, Shenzhen, China) equipped with a 7.5 MHz linear transducer was used to assess the ovarian status at the following time: (i) previous to superovulatory treatments to quantify the number of small (2–4 mm), large (>4 mm) and total follicles; (ii) onset of oestrous behaviour to quantify the large follicles (>4 mm); and (iii) 7th day of the oestrous cycle to quantify the number of *corpora lutea* (CL) and persistent follicles (>8 mm). Does were maintained in a standing position in all procedures. B-mode and colour flow Doppler videos of the ovaries were recorded by the same operator for further analyses. The occurrence of premature regression of corpus luteum (PRCL) was also evaluated at the CL count time (7th day of the oestrous cycle) by varying obtained in echogenicity and luteal vascularization. Afterwards, CL counting, 37.5 μg d-cloprostenol IM (Prolise®, Tecnopec LTDA, São Paulo, Brazil), was administered to induce CL luteolysis in both treatments.

Indexes of superovulatory response

The following variables for each doe were recorded after both treatments (eCG and FSH): number of small, large and total follicles previously to the superovulatory treatment, number of large follicles at oestrous onset, number of CL and persistent follicles on 7th day of the oestrous cycle.

A CL cut-off was lineate to divide the total number of does into the different ovulatory responder groups, after both hormonal treatments. According the median of number of CL per treatment, does were grouped into high or low ovulatory responders to eCG (high ≥7; low <7 CL) and to FSH treatments (high ≥23; low <23 CL), in order to evaluate the recurrence rate between eCG and FSH treatments.

Selection of animals to assess the follicular status influence at the onset of superovulatory treatments on ovulations

The cut-off point for entry in this analysis was the arithmetic average of the number of large follicles obtained at beginning of each treatment (eCG: 5.4; FSH: 4.4). Thus, a total of six goats (from 20 goats) were included with less than five and four large follicles at the beginning of the treatments with eCG and FSH, respectively, for new correlation between treatments, under low influence of large follicles.

Statistical analysis

Statistical analyses were performed using a computer software (SAEG® 9.0, Universidade Federal de Viçosa, Minas Gerais, Brazil). Simple linear and quadratic regression analysis and Pearson's correlation coefficient

were applied to assess the relationship between the following: (i) the follicular status before eCG or FSH dose and the number of CL obtained per treatment, (ii) the number of large follicles at oestrous onset and the number of CL obtained per treatment, (iii) the number of CL obtained in eCG treatment and FSH treatment and (iv) the number of CL obtained in eCG treatment and FSH treatment (from 6 goats, as described in 2.6.). The Spearman's rank correlation coefficient and Kappa coefficient were used to evaluate the relationship between high or low ovulatory responder does after each treatment. The intraclass correlation coefficient was adopted to assess the repeatability of the number of large follicles found at oestrous onset with the number of CL in response to each treatment. Differences in the occurrence of PRCL and persistent follicles, between treatments, were assessed by Fisher's exact test. For all tests, $p < 0.05$ was considered as statistically significant.

Results

Follicular status and number of corpora lutea obtained between treatments

The assessment of follicular population at the moment of eCG administration showed a mean of 5.4 ± 1.3 small follicles (range: 1–10), 5.3 ± 1.3 large follicles (range: 4–9) and 10.7 ± 2.7 total follicles (range: 7–16). Regarding to the FSH treatment, a mean of 11.0 ± 1.3 small follicles (range: 3-20), 4.4 ± 0.5 large follicles (range: 2–8) and 15.3 ± 1.5 total follicles (range: 8–28) was obtained. A significant lower number of CL was obtained in eCG-treated (9.1 ± 2.3) than FSH-treated does (26.2 ± 1.2). The occurrence of PRCL did not differ ($p > 0.05$) between eCG (20%; 4/20) and FSH treatment (30%; 6/20). Lastly, the frequency of persistent follicles at CL counting differed ($p < 0.05$) between eCG (30%; 6/20) and FSH treatment (90%; 18/20).

Correlations between the follicular status and number of corpora lutea obtained between treatments

Four and six does showed PRCL after treatment with eCG and FSH, respectively. Data from these goats were not used in the following correlations. As noted in Table 1, several relationships between follicular population and number of CL were found in both treatments that had a synergic effect when they were considered together. Importantly, positive correlations were obtained between the number of small follicles at first eCG/FSH dose with the number of CL (r ~0.47; $p < 0.05$) as well as the negative correlation between the presence of large follicles at eCG dose and number of CL ($r = -0.444$; $p < 0.05$). Furthermore, positive correlations were also obtained between the number of large follicles at oestrous onset with the number of CL (eCG: $r = 0.51$; $p < 0.05$/FSH: $r = 0.70$; $p < 0.05$).

Table 1. Pearson correlations obtained per treatment (eCG/FSH) or both treatments between the number of CLs and follicular data in goats

Number of CLs	eCG (n = 16)	FSH (n = 14)	eCG + FSH (n = 30)
Small follicles at start of treatment	0.490**	0.451**	0.701*
Large follicles at start of treatment	−0.444**	−0.100	−0.239***
Total follicles at start of treatment	0.177	0.380***	0.600*
Large follicles at oestrous onset	0.508**	0.704*	0.941*

CLs, corpora luteas; eCG, equine chorionic gonadotropin; FSH, follicle stimulation hormone.
*p < 0.01, **p < 0.05. ***p < 0.10.

A lower intraclass correlation coefficient was observed between the number of large follicles at the oestrous onset and the number of CL in the treatment with eCG (icc: 0.44; $p < 0.05$) when compared to treatment with FSH (icc: 0.86; $p < 0.05$).

As shown in Fig. 1, a significant positive relationship between the number of small follicles at the start of superovulatory treatment and large follicles at oestrous onset with the number of CL in the FSH treatment was obtained.

In Table 2, the simple and multiple linear equations are presented to estimate the number of CL from follicular data in both protocols (n = 30). A good multiple correlation between all collected follicular indexes (small follicles, large, total at the beginning of superovulation and large follicles at oestrous onset) was obtained for predicting the number of CL (r^2=0.86; $p < 0.05$). However, only the presence of the number of large follicles at oestrous onset was enough to estimate the number of CL (r^2=0.87; $p < 0.05$). The assessment of the follicular status, prior to superovulatory stimulus, was able to estimate approximately 50% (r^2=0.48; $p < 0.05$) in multi-ovulatory response. Moreover, the large follicles contributed negatively for the equation. Lastly, only the number of small follicles in the formula was effective to achieve the same index (r^2=0.49; $p < 0.05$).

When analysing ovulation response to both eCG and FSH treatments, in all does (n = 20), a significant relationship (r^2=0.498; $p < 0.05$) between the number of CL in response to each treatment was obtained. However, these data did not show a linear behaviour; just a quartic regression was efficient to explain this relationship (Fig. 2). From the correlation analyses of pre-selected goats (n = 6), with a lower number of large follicles prior to hormonal stimulation, a linear equation was obtained between treatments ($r = 0.76$; $y = 34.98–0.54x$; $p = 0.07$).

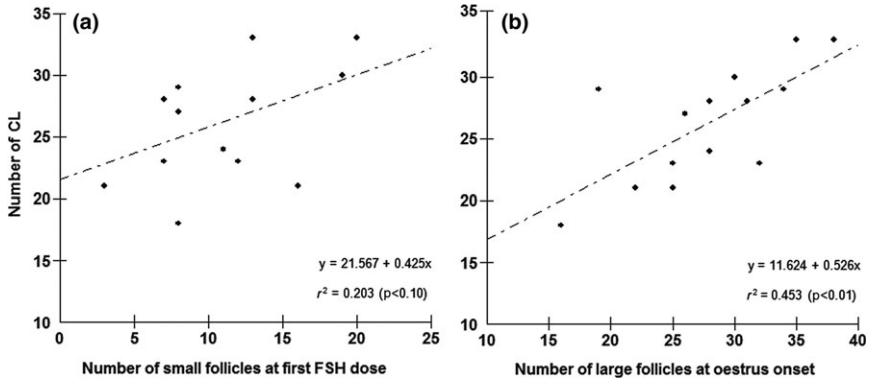

Fig. 1. Relationship between (a) the number of small follicles at first follicle stimulation hormone (FSH) dose; and (b) the number of large follicles at oestrous onset in FSH treatment with the number of *corpora lutea* in goats

Table 2. Simple and multiple linear equations to estimate the number of corpora lutea from follicular data at the beginning of superovulatory eCG and FSH treatments and at oestrous onset in goats

Follicles	Equations (n = 30)	r^2 ($p < 0.05$)
SFOL + LFOL + TFOL + LFOLE	$CL = 3.89 + 0.21_{FOLG} + 0.0_{FOLT} + 0.85_{FOLGE} - 0.22_{FOLP}$	0.86
SFOL + LFOL	$CL = 6.97 + 1.48_{FOLP} - 0.54_{FOLG}$	0.48
SFOL	$CL = 4.09 + 1.51_{FOLP}$	0.49
TFOL	$CL = 1.27_{FOLT} - 0.004$	0.36
LFOLE	$CL = 4.28 + 0.77_{FOLGE}$	0.87

SFOL, small follicles; LFOL, large follicles; TFOLT, total follicles; LFOLE, large follicles at oestrous onset.

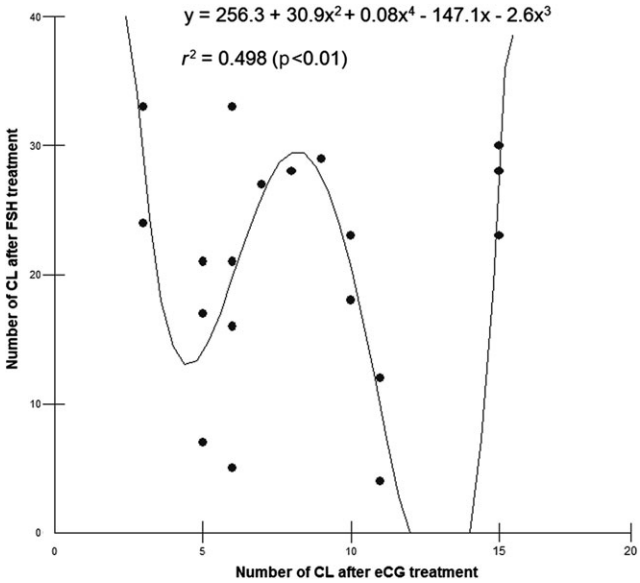

Fig. 2. Relationship between the number of corpora lutea in response to either equine chorionic gonadotropin or follicle stimulation hormone treatments in goats

Correlations between the stipulated cut-off point for each treatment

In this study, the recurrence rate was defined as the superovulatory ovarian response after FSH administration in relation to prior response to eCG treatment. Eight does of eleven with high ovulatory response to eCG still showed high ovulatory response after FSH treatment (72.7%; 8/11). Six does of nine with low ovulatory response to eCG treatment still showed low ovulatory response after FSH treatment (66.7%; 6/9). Overall, 14 does of six still showed high or low response after FSH treatment (70%; 14/20). The Kappa ($\kappa = 0.40$; $p < 0.05$) coefficient did show a moderate concordance regarding to donors to be classified as high or low responders between treatments. However, the Spearman coefficient (rs=0.39; $p = 0.08$) has just indicated a trend of relationship between them.

Discussion

The assessment of ovarian follicular population at eCG or first FSH dose showed high interindividual variability. Reasons for these findings could be caused by intrinsic factors, such as the number of primordial follicles at birth, genetic mechanisms, different hormonal doses or by external factors like environmental conditions and nutrition (Mossa et al. 2007). Nevertheless, other studies have shown similar means in the number of small and total follicles in goats (Cueto et al. 2006; Nogueira et al. 2015). Regarding the mean number of CL obtained per treatment, there have been reported similar means after either eCG or FSH superovulatory treatment (Senthil Kumar et al. 2003). In contrast, other authors reported a lower mean even after the use of a higher dose of eCG (1500 IU) (Holtz et al. 2012).

The use of eCG for superovulation treatments has been associated with a greater occurrence of PRCL than FSH treatments (Riesenberg et al. 2001; Senthil Kumar et al. 2003). However, this was not observed in the present study (eCG: 20% *vs*. FSH: 30%), even after the single dose of hCG (500 IU) in FSH-treated goats, as recommended previously by Saharrea et al. (1998). More recently, Shabankareh et al. (2012) suggested with promising results a single dose of hCG (500 IU) when starting a superovulatory treatment and in the following 2 days.

The greater frequency of persistent follicles in FSH-treated compared with eCG-treated does may be explained by the use of hCG. As this hormone has a long half-life (39.4 h; Saleh et al. 2012) and was administered after end of oestrus, it may have continued to stimulate follicle growth from the subsequent follicular wave culminating with greater number of large follicles quantified on 7th day of the cycle.

The current study has found a significant relationship between the numbers of small and total follicles at eCG and at first FSH dose with the number of CL in response to treatments. These positive relationships have been found in ewes (González-bulnes et al. 2004) or not (Bruno-Galarraga et al. 2015). Similarly, the importance of the start of a superovulatory treatment in the presence of a pool of emerging follicles was already demonstrated in goats (Menchaca et al. 2002, 2007). However, to the authors' knowledge, this is the first report that demonstrates a positive significant relationship ($r = 0.70$; $p < 0.01$) between the number of small follicles at superovulation time with the response of the goat donor, regardless of the source of hormonal stimulation (eCG or FSH). It was also proposed an equation ($y = 21.57 + 0.43x$) that can help to predict the superovulatory response by FSH in goats from the prior ultrasound assessment of the number of small follicles.

The negative relationship was found between the number of large follicles at superovulation time and the number of CL, in agreement with previous studies in Alpine goats [5–6] and Merino ewes (Bruno-Galarraga et al. 2015) under subtropical conditions. Therefore, it highlights the importance of the starting a superovulatory treatment in the absence of large follicles. Moreover, the number of large follicles at oestrous onset in FSH treatment has demonstrated better correlations with the number of CL than in eCG treatment. The fact can be clarified by short half-life of FSH (5 h–cattle; Laster 1972) and its synchronized stimulation of a pool of follicles unlike longer biological action of eCG (40 h - cattle; Dieleman et al. 1993). This is an important tool when there is the need to check whether the goat donor will be responding or not to FSH superovulatory treatment 7 days before counting of CL. In this way, it was proposed an equation ($y = 4279 + 0{,}776x$; $r^2=0.87$; $p < 0.01$) that can help to estimate the number of ovulations from the counting of large follicles at oestrous onset after superovulatory treatment.

In the current study, from the total number of experimental goats (n = 20), it was not found a linear behaviour in the relationship between the number of CL obtained in response after each treatment (eCG vs FSH). Just a quartic regression was able to partially elucidate ($r^2=0498$; $p < 0.01$) the relationship between them. However, when it was performed the pre-selection of goats that had less influence of large follicles at the beginning of superovulatory protocols, a linear correlation ($r^2=0.48$; $p = 0.07$) was obtained. Thus, it was proved the existing bias in carrying out the screening test with eCG under presence of large follicles. Using a similar methodology adopted in this study, other authors also demonstrated a linear correlation response ($r^2=0.63$) to FSH from the previous screening test with eCG in sheep (Bruno-Galarraga et al. 2015). However, in their research, sheep had a lower number of large follicles as compared to those observed in the present study in goats. Thus, it is important to consider the role of follicular status and the differences between small ruminants (goats and sheep) on superovulatory responses. Furthermore, it is suggested to synchronize follicular emergency in goats when adopting the screening methodology proposal, especially in the breeding season.

The stipulated cut-off point from the median, measure of central tendency, was effective in the identification of 70% (14 of 20) of goats with high or low potential ovulatory response. Other authors have indicated the even division of the total number of animals as the cut-off point for Merino sheep with high or low potential ovulatory response reaching a 84% (26 of 31) of recurrence rate (Bruno-Galarraga et al. 2015). Thus, different statistical methodologies can be adopted to obtain these indices in small ruminants.

The follicular status, in particular, the presence of large follicles prior to the beginning of superovulation treatments unfavourably influenced multiple ovulation rate. The positive relationship found between the number of small follicles and superovulatory response highlights the importance to synchronize follicular emergency in goats when performing multiple ovulation treatments.

It was concluded that the screening protocol with eCG has the potential to identify Saanen goats that will better respond to the FSH treatment. In addition, it highlighted the importance of an ultrasound assessment prior to the beginning of superovulation treatments with FSH in order to characterize the follicular status and identify potential donors with probable high ovulatory response for MOET programmes in goats.

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Conflict of interest

None of the authors have any conflict of interest to declare.

Author contributions

We declared that all authors have made substantial contributions to the research and article as follows: Balaro contributed to design and data acquisition of the experiment, analysis and to drafting the article; Maia contributed to data acquisition of the experiment and revision of the article; Brandão, Souza-Fabjan, Cueto and Gibbons and Fonseca contributed to the conception and design of the experiment, data interpretation and final revision of the article. This work was carried out at Coronel Pacheco, Minas Gerais, Brazil.

Highlights

The follicular status prior to superovulation affects multiple ovulation rate.
eCG has the potential to identify goats response to the FSH superovulatory treatment.
The synchronization of follicular emergency in goats may improve the ovulation rate.
Ultrasonography is an essential tool to identify goats of high ovulatory response.

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