



## Effectiveness of different hCG and GnRH based protocols in progesterone primed goats on estrus induction and reproductive outcomes in out-off-season goats

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### ABSTRACT

We evaluated whether Gonadotrophin releasing hormone incorporation to the Human chorionic gonadotropin protocol improves the sexual response during the natural anestrus season in goats. Thirty-two adult multibreed anovulatory goats (n = 8, four groups), received 20 mg progesterone i.m. on d-1; on d0, all goats received 7.5 mg prostaglandin F i.v. and control group (CG) 0.5 mL of saline i.m., hCG group (GH) 100 IU of hCG i.m., hCG+GnRH group (GN) 100 IU hCG i.m. + 8.4 µg of GnRH at once, and hCG+GnRH-24h group (GN24) 100 IU hCG i.m. + 8.4 µg of GnRH 24 h apart. GN, GN24 and GH depicted estrus and ovulatory activity, favoring the GH (25%, 25% and 100% for both variables, respectively). Regarding pregnancy rate, the largest values (P<0.05) were observed in GH (12%, 12% vs 75%, respectively). A protocol based on P<sub>4</sub>+PGF+hCG was the best option to induce and synchronize estrus as well as ovulation regarding the administration of GnRH during the natural anestrus season in goats.

**Keywords:** Goats, anestrus season, estrus induction protocols, GnRH, hCG, reproductive outcomes

Many breeds of goats depict seasonal reproductive activity generating a seasonal offering of their main products, milk and meat, a productive scenario which affects not only the producers but also the consumers (Gómez-Brunet *et al.* 2011). In the arid lands from Northern Mexico, goat farmers under extensive conditions seek out to breed does during the anestrus season to satisfy the such shortage in the production cycle (Ángel-García *et al.* 2014). The manipulation of reproduction periodicity in goats is an important tool (Argüello, 2011), and different hormonal protocols have been developed to induce out-off-season reproductive activity using different progestogens, prostaglandins and gonadotropins (Abecia *et al.* 2012). Besides, such hormonal protocols have been essential in the development of different assisted reproductive technologies (Paramio and Izquierdo, 2014).

Gonadotropins analogues such as eCG and hCG, have been used in different protocols to synchronize the

reproductive activity during the natural breeding season as well as to induce and to synchronize reproductive function during the natural anestrus season. In addition, such protocols have been used to increase ovulation rate (Omontese *et al.* 2013). Yet, during the anestrus season, these protocols also include the use of progesterone (P<sub>4</sub>) in order to sensitize the hypothalamic-pituitary-gonadal axis to promote a better response to the gonadotropic stimuli and to generate the release of gonadotropins; LH and FSH (Maffili *et al.* 2006). Recently, as demonstrated by our group, application of 25 mg P<sub>4</sub> i.m. followed by 250 IU of eCG induced reproductive activity in goats during the natural anestrus season (Rodríguez-Martínez *et al.* 2013).

Both GnRH or analogs have been used mainly in cattle and sheep in order to improve estrus synchronization and ovulation. When applied prior to or at the moment of gonadotropin administration, it is possible to prevent a premature increase in LH while improves in parallel,

the quality of the oocyte, increasing fertility rate (Copperman and Benadiva, 2013). Administration of GnRH or analogues from 24 h and up to 12 days after natural breeding or insemination has been another strategy to induce either luteinization or ovulation of persistent follicles. This approach eliminates a possible source of estradiol to prevent the onset of a premature luteolysis. Besides, it has been also used prior to the maternal recognition of pregnancy process, in order to compensate a possible luteal insufficiency as well as to stimulate embryo development by amplifying the embryonic signal at very early pregnancy stages (Peters, 2005).

However, while some studies support the idea that the use of GnRH is meaningless, others propose a decrease in fertility rate (Akar *et al.* 2014; Baki-Acar *et al.* 2013; Saribay *et al.* 2012). Therefore, it is important to define the most accurate time for GnRH or analogues to be applied in order to increase the reproductive induction response, especially in goats, since plenty of information is available in cattle and sheep. Considering such rationale, the aim of this study was to determine if the inclusion of GnRH to the hCG protocol improves the reproductive outcomes in goats during the anestrus season.

## MATERIALS AND METHODS

All the methods used in this study were in accordance with accepted international guidelines (FASS, 2010).

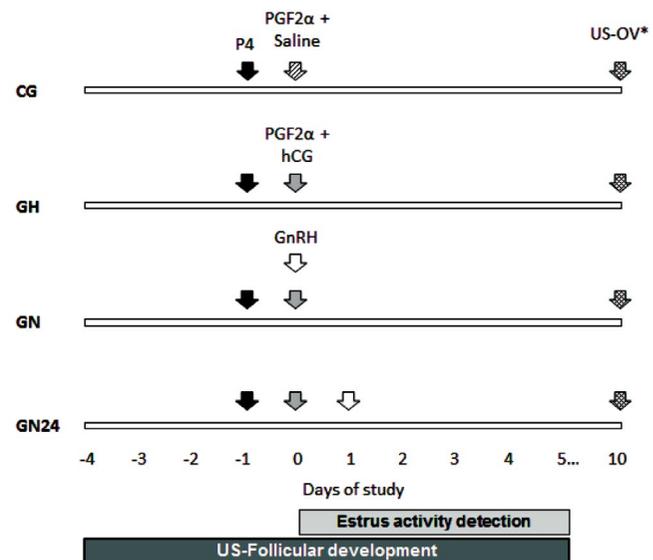
### Location, animals and experimental design

The study was conducted from April 28 to May 15, the natural anestrus season, in the Ejido Santa Fe, Torreon, Coahuila, Mexico, 25°33'48.24" North, 103°20'08.59" West, at an altitude of 1,120 m. The area is characterized by a dry climate, with annual averages for temperature and precipitation of 23.1 °C and 230 mm, respectively. The goat anestrus status was confirmed throughout ultrasonographic transrectal scanning (Aloka SSD 500, Richmod, BC, Canada) as previously described (De Santiago-Miramontes *et al.* 2011).

Thereafter, adult anovulatory multibreed goats (n=32) were divided in four experimental groups (n=8 per group) with homogeneous live weight ( $40.0 \pm 7.0$  kg) and body condition score ( $1.7 \pm 0.3$  units). At the beginning of the study, goats were randomly placed in pens (4 x 4 m) 50 m

apart from each other, exposed to natural environmental temperature and photoperiod. On day -1, all goats received 20 mg (i.m.) of P<sub>4</sub> and, on day 0, all goats received 7.5 mg (i.v.) of PGF<sub>2α</sub> (Dinoprost, tromethamine). Thereafter, each pen was randomly assigned to one of four experimental treatments: 1). Control Group (CG) receiving 0.5 mL i.m. of saline on d0, 2). GH-Group (GH) receiving 100 IU i.m. of hCG on d0, 3). GN-Group (GN) receiving 100 IU i.m. of hCG + 8.4 µg of GnRH at once, on d0, and 4). GN-24-Group (GN24) receiving 100 IU i.m. of hCG on d0 and 8.4 µg of GnRH 24 h apart.

Goats were fed alfalfa hay plus 200 g of a commercial concentrate (14% CP) with free access to mineral salts and water. Goats remained under these conditions until the experimental protocol was concluded; thereafter, goats returned to the extensive-range land production system. A schematic representation of the experimental protocol with the four experimental treatments is presented in Figure 1.



\*US-OV: ultrasound to detect ovulation

**Fig. 1:** A schematic representation of the experimental protocol according to experimental groups of adult multiracial anestrus goats induced to estrus during the natural non-breeding season in Northern Mexico

### Measured variables and statistical analyses

Follicular dynamics was evaluated on days -4 to +5 according to the protocol proposed by Medan *et al.*

(2005); in brief, once the ovaries were located, specific diagram from each ovary registering both localization and development of the follicles were drawn. Estrus activity was determined twice per day (0600 am and 1800 pm), from day 0 to day 5 using two sexually active mixed-breed males. While estrus activity was confirmed when the female accepted to be mounted by the male, ovulatory activity was confirmed on day 10 through transrectal ultrasonographic scanning. Ovulation activity was confirmed by the presence of at least one corpus luteum in either ovary (Simoes *et al.* 2008). Thereafter, pregnancy was diagnosed on day 45 throughout ultrasonographic scanning. At kidding, the number of kids born per kidding female was recorded. The percentage of goats depicting estrus and ovulation was compared by using the Exact Fisher test. The evaluation of follicular diameter considered the “T” test, MYSTAT (Version 12, 2007). All the analyses considered a 95% confidence interval.

**Table 1:** Means ( $\pm$ SEM) and percentages for different reproductive outcomes in adult multiracial goats exposed to different estrus inducing protocols in Northern Mexico

Variables	CG	GH	GN	GN24
Estrus (%)	0	100% (8/8) <sup>a</sup>	25% (2/8) <sup>b</sup>	25% (2/8) <sup>b</sup>
Ovulation (%)	0	100% (8/8) <sup>a</sup>	25% (2/8) <sup>b</sup>	25% (2/8) <sup>b</sup>
Ovulation rate (n)	—	1.8 $\pm$ 0.2 <sup>a</sup>	1 $\pm$ 0.1 <sup>b</sup>	1.5 $\pm$ 0.5 <sup>a</sup>
Estrus latency (h)	—	65 $\pm$ 4 <sup>b</sup>	66 $\pm$ 18 <sup>a</sup>	66 $\pm$ 18 <sup>b</sup>
Latency from estrus to ovulation (h)	—	33 $\pm$ 7 <sup>a</sup>	54 $\pm$ 18 <sup>b</sup>	30 $\pm$ 6 <sup>a</sup>
Latency from d0 to ovulation (h)	—	93 $\pm$ 4,5	108 $\pm$ 0	84 $\pm$ 12
Estrus duration (h)	—	39 $\pm$ 12 <sup>b</sup>	54 $\pm$ 18 <sup>a</sup>	30 $\pm$ 6 <sup>b</sup>
Gestation (%)	—	75% (6/8) <sup>a</sup>	12% (1/8) <sup>b</sup>	12% (1/8) <sup>b</sup>
Prolificacy (n)	—	1.5 $\pm$ 0.2 <sup>a</sup>	1.5 $\pm$ 0.5 <sup>a</sup>	1 <sup>b</sup>
Follicular diameter (mm)	—	7.7 $\pm$ 0.3 <sup>a</sup>	9.5 $\pm$ 0.5 <sup>a</sup>	6.5 $\pm$ 0.6 <sup>b</sup>

<sup>a,b</sup> Among columns, means without a common superscript, differ ( $P < 0.05$ )

CG; 0.5 mL i.m. of saline, GH; 100 IU i.m. of hCG, GN; 100 IU i.m. of hCG + 8.4  $\mu$ g of GnRH at once, and GN24; 100 IU i.m. of hCG + 8.4  $\mu$ g of GnRH 24 h apart

## RESULTS AND DISCUSSION

Results of this study do not support our working hypothesis that inclusion of GnRH to the hCG induction protocol

would improve the reproductive outcomes of goats during the natural anestrus season. hCG has been used as one of the most potent stimulators of the final oocyte maturation because of its marked structural and biologic similarities with LH (Humaidan *et al.* 2012). In our study, the GH-group depicted the most reduced number of middle follicles on day +3 regarding day 0, with an increase in the number of the largest follicles on day +3. According to Scaramuzzi *et al.* (2011), both primary follicles or small follicles are not LH-responsive, yet, middle and large follicles are LH-dependent for their growth. Considering the high similitude of actions between hCG and LH, a potential scenario is that the middle follicles were influenced by hCG-administration, increasing their size while generating an increased estrus and ovulatory response.

Table 1 concentrates the reproductive outcomes of the different experimental groups. Goats from the CG-group never depicted either estrus nor ovulation. On the contrary, goats from the groups GN, GN24 and GH depicted estrus activity and ovulatory activity, yet, favoring to the GH-groups (25%, 25% and 100% for both variables, respectively). The same was true regarding pregnancy rate, with the largest values ( $P < 0.05$ ) observed in the GH-group (12%, 12% vs 75%, respectively).

The GnRH treated females at the time of hCG administration were negatively influenced since they depicted a decreased estrus and ovulatory response (25%) while a reduced pregnancy rate (12%). Such reproductive outcomes disagree with other authors (dos Santos-Cavalcanti *et al.* 2012; Rekik *et al.* 2014; Titi *et al.* 2010), who demonstrated that the use of GnRH with other hormones is quite effective to induce and synchronize the LH surge and the ovulatory response as also suggested by Pierson *et al.* (2003).

Yet, the positive effect of exogenous GnRH administration may promote variations in such response depending on the stage of the estrous cycle of the treated females. Presumably, the injection of GnRH induced the LH surge which in turn may have promoted either ovulation or follicular atresia (Husein *et al.* 2005). Therefore, it is tempting to speculate that the large follicles may have regressed, since at the moment of the GnRH injection, the GN-group depicted an increased number of large follicles ( $> 6$  mm). Such scenario suggests a lack or reduced response to the LH surge. Therefore, the ovulatory failure could be due to

**Table 2:** Means ( $\pm$ SEM) of small, medium and large follicles (n) on days 0, +3 and +5 in adult multiracial goats exposed to different estrus inducing protocols in Northern Mexico

Follicles	CG			GH			GN			GN24		
	d 0	d +3	d +5	d 0	d +3	d +5	d 0	+3 d	d +5	d 0	d +3	d +5
Small $\leq$ 3 mm (n)	2,1 $\pm$ 0,7 <sup>a</sup>	3 $\pm$ 1,0 <sup>a</sup>	3,1 $\pm$ 0,9 <sup>a</sup>	0,7 $\pm$ 0,5 <sup>a</sup>	1,2 $\pm$ 0,4 <sup>a,b</sup>	2,6 $\pm$ 0,7 <sup>b</sup>	3,8 $\pm$ 0,8 <sup>a</sup>	0,6 $\pm$ 0,3 <sup>b</sup>	2,6 $\pm$ 0,7 <sup>a</sup>	5,4 $\pm$ 0,9 <sup>a</sup>	7,4 $\pm$ 1,2 <sup>a</sup>	4,1 $\pm$ 1,2 <sup>a</sup>
Medium 4-5 mm (n)	2 $\pm$ 0,4 <sup>a</sup>	2,9 $\pm$ 0,5 <sup>a</sup>	2,5 $\pm$ 0,4 <sup>a</sup>	3,2 $\pm$ 0,4 <sup>a</sup>	1,6 $\pm$ 0,3 <sup>b</sup>	1,4 $\pm$ 0,4 <sup>b</sup>	2 $\pm$ 0,5 <sup>a</sup>	1,9 $\pm$ 0,5 <sup>a</sup>	0,5 $\pm$ 0,2 <sup>b</sup>	3 $\pm$ 0,7 <sup>a</sup>	2,1 $\pm$ 0,3 <sup>a</sup>	2,8 $\pm$ 0,5 <sup>a</sup>
Large $\geq$ 6 mm (n)	2 $\pm$ 0,4 <sup>a</sup>	1,6 $\pm$ 0,4 <sup>a</sup>	1,3 $\pm$ 0,3 <sup>a</sup>	1,2 $\pm$ 0,4 <sup>a</sup>	2,4 $\pm$ 0,4 <sup>b</sup>	0,8 $\pm$ 0,3 <sup>a</sup>	1,4 $\pm$ 0,4 <sup>a</sup>	2,7 $\pm$ 0,6 <sup>a</sup>	0,8 $\pm$ 0,3 <sup>a</sup>	0,7 $\pm$ 0,3 <sup>a</sup>	0,3 $\pm$ 0,2 <sup>a</sup>	0,5 $\pm$ 0,1 <sup>a</sup>

<sup>a,b</sup> Among treatments, means without a common superscript, differ ( $P < 0.05$ )

CG; 0.5 mL i.m. of saline, GH; 100 IU i.m. of hCG, GN; 100 IU i.m. of hCG + 8.4  $\mu$ g of GnRH at once, and GN24; 100 IU i.m. of hCG + 8.4  $\mu$ g of GnRH 24 h apart

an asynchronous follicular growth presumably as a result of a dysfunction in the LH receptors during their initial development stages at the moment of the endogenous LH surge.

The GN-group depicted the largest estrus duration, an increased estrus latency from estrus to ovulation and from day 0 to ovulation as well as the lowest fertility rate regarding the other experimental groups. According to Saharrea *et al.* (1998), GnRH administration around the estrus onset diminished the presence of non-ovulated large follicles probably due to a GnRH refractory action by the pituitary cells after an LH endogenous release. In bovine, a reduced response to GnRH has been demonstrated when is administrated after the LH surge occurred. On this respect, in sheep, Kaya *et al.* (2013) reported that during seasonal anestrus, the hypothalamus is quite sensitive to the negative feedback from estradiol. During this period of time, LH is released in a less frequency fashion, generating the lack of both estrus and ovulation. Therefore, gonadotropins are required to support growth of both small and medium sized follicles, although estrus occurrence and fertility indices may fluctuate depending on the synchronizing method. Our results are in line with Moghaddam *et al.* (2012), who reported that GnRH administration 24 hours after P<sub>4</sub> withdrawal did not affect estrus induction, ovulation or fertility. Considering such findings, it is possible to suggest that GnRH may had enlarged the lifespan of the mature follicles generating their demise.

The GH and GN24 groups depicted the largest ovulation rate, a decreased latency to estrus while a decreased estrus

duration. As reported by (Husein *et al.* 2005), after GnRH administration, it is possible to observe a new follicular wave generating the development of a large follicle able to release enough estradiol escorted with the presence of a standing estrus. In the GH24 group, the large follicles diminished on day +3, while augmented on day +5. As proposed by Oliveira *et al.* (2009), GnRH administration induce a pituitary discharge of LH followed by ovulation of the dominant follicle and the regression of the small ones, generating the emergency of a new follicular wave, a physiological scenario that may had occurred in the GH24 group.

Both estrus response and pregnancy rate favored to the hCG-group. Our results are superior than a previous study where treated females depicted a 75% estrus response but with a decreased interval to estrus (46 h) (Fonseca *et al.* 2005); this scenario could be motivated because of the source of P<sub>4</sub>. Regarding estrus induction, our results are also superior than those values reported by Fonseca *et al.* (2005) who obtained interesting outcomes with 96% estrus response, a reduced estrus duration (20.7 h), a decreased interval to estrus (48 h), a minor estrus duration (20.7 h), yet with a similar (77%) pregnancy rate. The last suggests that hCG represents a viable option to induce and synchronize estrus during the out-off-season in goats.

Table 2 concentrates the observed follicular diameters on days 0, 3 and 5. In general, there was a trend to observe the largest number of small follicles in the CG and GN24 while there was observed no difference ( $P > 0.05$ ) among experimental groups regarding the number of medium

and large follicles; as the time advanced, the follicular diameter increased in all groups.

The Control group never depicted any reproductive response. Such null reproductive outcome agrees with Nogueira *et al.* (2015) which, when evaluating follicular waves and hormone profiles, confirmed seasonal anestrous in Boer goats during the non-breeding season under tropical conditions. Besides, and not in line with the findings reported by Sharma and Purohit (2009), administration of P<sub>4</sub> alone was not able to induce estrus behavior during the anestrous season.

To conclude, a protocol based on P<sub>4</sub> +PGF +hCG was the best option to induce and synchronize estrus as well as ovulation with respect to the inclusion of GnRH during the natural anestrous season in goats. Further research is required to evaluate different doses and time of GnRH administration in order to improve reproductive outcomes during the out-off-season in goat production systems.

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