Growth and reproductive traits of F1-generation transgenic goats for human granulocyte-colony stimulating factor

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Abstract. To ensure that animal welfare requirements and phenotypic characteristics of the newly produced transgenic lines are not compromised, an evaluation of all individuals is necessary. This can be inferred by the analysis of the growth and reproduction parameters. The present study was designed to determine the impact of the insertion of human granulocyte-colony stimulating factor (hG-CSF) transgene on growth and reproductive characteristics in first-generation (F1) goats from two transgenic lines. Bodyweight (BW) development (BW at birth, mean BW gain before weaning, BW at weaning, mean BW gain after weaning, BW at puberty), as well as reproductive parameters (age at puberty, ejaculate volume, concentration, total sperm per ejaculate, massal motility, progressive individual motility, major and minor defects) were similar ($P > 0.05$) between transgenic (T) and non-transgenic (NT) goats. Significant ($P < 0.05$) differences in mean ($\pm$s.d.) white blood cell count were observed between T and NT in first day of life ($174.6 \pm 14.7 \times 10^3$ and $15.0 \pm 4.0 \times 10^3$ cells/µL), and during ($66.8 \pm 21.1 \times 10^3$ and $17.0 \pm 4.6 \times 10^3$ cells/µL) and after ($36.6 \pm 4.0 \times 10^3$ and $15.5 \pm 2.2 \times 10^3$ cells/µL) suckling, even though hG-CSF has not been detected in blood serum in any analysis. Although other cell counts were occasionally higher in T animals, differential counts showed that this difference was mainly due to an increased number of neutrophils, which represents 84.6%, 67.2% and 56.8% of total white blood cell count respectively, in the three time periods. Kidney and liver biochemical analyses indicated that all goats were healthy. Thus, it is possible to assume that all animals are normal and had no deleterious effects on either growth or reproductive parameters by the presence of transgene or as a consequence of leukocyte profile alteration.

Additional keywords: neutrophilia, puberty, welfare, white blood cell.

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Introduction

The production of transgenic farm animals containing exogenous DNA stably incorporated into their genome, when able to be transmitted to their offspring in a Mendelian fashion, has several applications. Besides the obvious scientific interest for the study of genes and their regulation, transgenic animal technologies have been proposed as a method to accelerate livestock improvement, by means of introducing new genes or modifying the expression of endogenous genes that regulate traits of economic importance (Wheeler 2003) and the production of recombinant proteins (Lavine 2009).

Although there is a continuous and remarkable development in transgenic technology, the consequences of genotypic changes induced by this type of experimental mutation cannot be completely predicted. This is especially true for the majority of transgenic animals that are generated by pronuclear injection. Randomly integrated foreign DNA may increase the risk of disruption of endogenous genes (Jackson *et al*. 2010) and disturb normal physiological processes, possibly resulting in discomfort, which is crucial to animal welfare (Mertens and Rulicke 2007). Additionally, an inappropriate control of transgene expression could lead to overexpression or expression in undesirable tissues (Pursel *et al*. 2004). Thus, several studies have reported physiological (Pursel *et al*. 1990) and reproductive (Maleszewski *et al*. 1998; Bryła *et al*. 2010) disorders in transgenic animals.

Certainly, the health and welfare of transgenic animals raised for production purposes is of central importance to potential consumers and producers (Jackson *et al*. 2010). Animal welfare is a concept that involves both physical and subjective evaluations of individuals (Duncan 2005; Mertens and Rulicke 2007). The physical aspects of general health, as standard measures of body development and reproductive fitness, such as semen quality and age at puberty, can be used as indicators of transgenic-animal health (Jackson *et al*. 2010). Another key indicator of health in living organisms is their ability to grow from birth to adulthood in a way similar to young individuals of the same species and breed. Van Reenen (2009) suggested that

the study of a transgenic line should be performed not only during the period of transgene expression, but also at different stages of life, including pregnancy, birth, puberty and adulthood.

Our group reported the production of a couple of transgenic goats (10M and 12F lineages) containing human granulocyte-colony stimulating factor (hG-CSF) fused to goat α-S1 casein (CSN1S1) promoter produced by pronuclear injection (Freitas *et al.* 2012). This protein acts in the prevention of febrile neutropenia and, currently, is recommended for patients with substantial risks to stimulate the proliferation and differentiation of granulocytic lineage cells (Crea *et al.* 2009). The female founder (12F) successfully expressed the recombinant protein in her milk and at a concentration that was compatible with commercially viable investments (Moura *et al.* 2013). Moreover, the hG-CSF transgene was transmitted by Mendelian fashion to first-generation progenies (F1) from both lineages (Freitas *et al.* 2012; Moura *et al.* 2014). However, according to Mertens and Rulicke (2007), to ensure transgenic-animal welfare requirements, a careful phenotype characterisation and welfare assessment has to be undertaken routinely for each newly produced lineage, at individual and lineage level, starting by the standardised monitoring of founders and their consequent generations.

The present study was designed to evaluate the impact of the insertion of hG-CSF transgene in F1 goats derived from two transgenic lineages, in comparison with non-transgenic controls from their birth to 10 months old, on bodyweight development, reproductive parameters such as age at puberty and semen quality, leukocyte profile, hepatic and renal function analysis, and ectopic expression for the protein in blood serum.

Materials and methods

Animal ethics and biosecurity

All protocols used in the study were approved by the Committee of Animal Ethics (09144595-7/50) of the State University of Ceará and Brazil's Biosafety Technical National Committee (CQB 0228/06) to work with genetically modified organisms. Additionally, all experimental procedures were conducted according to the guidelines for the ethical use of animals in research (ASAB 2006).

Location and experimental animals

The experiment was conducted in the Laboratory of Physiology and Reproduction Control of the State University of Ceará, Fortaleza (3°43′47″S, 38°30′37″W, ~15 m above sea level). For F1 generation, animals from both transgenic (T) lineages (12F and 10M) were mated with non-transgenic (NT) goats. In total, nine experimental animals of Canindé breed were obtained and used, including females (T = 3, NT = 1) and males (T = 2, NT = 3). The hG-CSF transgene was detected in skin biopsies from the ears of 2-week-old founders by using polymerase chain reaction (PCR) amplification according to the method reported by Freitas *et al.* (2012). Briefly, the identification of transgenic animals carrying the DNA construct was conducted by PCR with the following two pairs of primers: PrA (forward) 5′-AAA GGA TAA GGCTAA TGA GG-3′ and PrB (reverse) 5′-CGT TGT ACT TTT GTA CTG AGC-3′, and PrC (forward) 5′-TCT GCA AAA GCA GGC TAA AGG-3′ and PrD (reverse)

5′-GCCAAG ACA CTC ACC CAT CAG-3′, which amplify respectively, products of 704 and 530 bp at the ends of the DNA construction. DNA samples were obtained by phenol–chloroform extraction according to the protocol described by Sambrook *et al.* (1989). The PCR amplifications were performed using 50 ng of genomic DNA, 2 μM of each primer and 0.25 U of GoTaq DNA polymerase (Promega, Madison, WI, USA). The thermal cycling was performed with the following steps: (1) 3 min at 95°C, (2) 35 cycles of 30 s at 95°C, 30 s at 55°C and 30 s at 72°C, and (3) 5 min at 72°C. The amplicons were analysed by electrophoresis on a 1.5% agarose gel and visualised by ethidium bromide staining. Amplification of the β-actin gene was performed as the reaction control. Additionally, genomic DNA samples from a non-transgenic goat and an hG-CSF transgenic goat (previously obtained by Freitas *et al.* 2007) were used as the negative and positive controls respectively.

Experimental conditions

After birth, all animals (T and NT) received suckling up to 3 months of age when weaning was performed. Before weaning, once a day for a period of 5 h, the animals were separated from their mothers and kept in another pen, where they had free access to water, Tifton (*Cynodon dactylon*) hay, commercial concentrate (Fri-Ribe, Teresina, Brazil), and mineralised salt licks. After this period, animal diets were supplemented with 0.2 kg/day of the concentrate mentioned above, with Tifton hay, water and mineralised salt licks *ad libitum*.

Assessment of bodyweight (BW) development

Goats were monitored weekly regarding their BW development from birth to 10 months of age by the use of an electronic scale adapted for the species (Leader, Araçatuba, Brazil). It is noteworthy that weight measurement was always performed during early morning, aiming to obtain the data before feeding the animals, maintaining a standard analysis of the values obtained.

Puberty detection in females

The determination of the onset of ovarian activity initiated at 3 months of age and continued until puberty, by plasma progesterone concentration analysis. Blood samples were collected from all female goats (T and NT) by jugular venipuncture using 4-mL tubes containing EDTA (BD Vacutainer, Becton Dickinson and Co., Holdrege, NE, USA), always in the morning, once per week. The tubes were placed on ice until centrifugation at 4400*g* for 15 min at 4°C. During this period, plasma was divided into two parts, one for biochemical analysis and the other for P4 analysis. Progesterone concentrations were measured using a commercial chemiluminescence specific kit (Immulite Siemens, Deerfield, IL, USA; sensitivity of 0.03 ng/mL). Progesterone concentrations greater than 1 ng/mL in two consecutive samples were considered as indicative of luteal activity, indicating the onset of puberty (Al-Hozab and Basiouni 1999).

Puberty detection in male and sperm analysis

The detection of puberty started at 3 months of age, when individual sexual behaviour was assessed once per week.

Table 1. Bodyweight (BW) parameters of F1-generation hG-CSF transgenic (T) and non-transgenic (NT) male and female goats before (up to 3 months old) and after (3–10 months old) weaning

Within a row, values with different letters represent a significant difference (at *P* = 0.05)

Parameter	T (*n* = 5)	NT (*n* = 4)
BW at birth (kg)	2.54 ± 0.29	2.34 ± 0.19
Mean BW gain before weaning (kg/week)	0.86 ± 0.09	0.83 ± 0.21
BW at weaning (kg)	13.72 ± 1.59	13.11 ± 2.26
Equation of BW gain before weaning (kg/week)	0.1236x + 2.43	0.1249x + 2.46
Mean BW gain after weaning (kg/week)	0.24 ± 0.32	0.20 ± 0.25
Equation of BW gain after weaning (kg/week)	0.0287x + 12.55	0.0287x + 10.28
BW at puberty (kg)	17.78 ± 3.03	14.90 ± 2.43

Males had a limit of 5 min to mount female goats induced to oestrus by weekly intramuscular injections of 2 mg oestradiol cypionate (ECP, Pfizer Animal Health, New York, NY, USA). After ejaculation, vaginal-smear slides were obtained and observed under a phase-contrast optical microscope (Eclipse E200, Nikon, Tokyo, Japan) for the presence of live sperm. The animals were considered pubescent when they presented the following sequences of sexual behaviour: penile exposure, mating, ejaculation and presence of mobile spermatozoa in the ejaculate (Delgadillo *et al*. 2007).

So as to verify the morphological and physical characteristics of the semen, at least five samples were collected from each buck aged 8–10 months old, through the artificial vagina method direct into graduated plastic tubes. After collection, semen was evaluated for volume, concentration, total sperm per ejaculate, massal motility, percentage of sperm cells with progressive individual motility and spermatic morphology (minor, major and total defects) according to Souza *et al*. (2011). All semen analyses were assessed through phase-contrast optical microscopy (Eclipse E 200).

Hematological and biochemical analysis

Blood samples were collected from all male and female goats (T and NT) by jugular venipuncture using 4-mL tubes containing EDTA (BD Vacutainer), always in the morning, once per week for white blood cell (WBC) and differential cell counts from birth to 10 months old. To determine the total WBC, an automatic analyser (CELL-Dyn 3700, Abbott Laboratories, Abbott Park, IL, USA) was used, whereas differential cell count was performed using a small drop of blood smeared on a clean microscope slide and then examined by Romanowsky staining (Nikon Eclipse E400). Conversely, blood serum biochemical analysis was performed every 15 days by the use of serum-separation tubes (BD Vacutainer), to determine urea, creatinine, glucose, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentrations (BT 3000 plus; Winer Laboratory, Rosario, Argentina). Tubes were placed on ice until centrifugation at 4400*g* for 15 min. Aliquots of plasma and serum were immediately sent to the laboratory for each assay.

hG-CSF analysis in blood serum

The same process was conducted for blood collection and serum sample obtention for hG-CSF quantifications in all T and NT

Table 2. Semen parameters from F1-generation hG-CSF transgenic (T) and non-transgenic (NT) goats

Semen was collected from all bucks once per week (five replicates). Within a row, values with different letters represent a significant difference (at *P* = 0.05)

Parameter	T (*n* = 2)	NT (*n* = 3)
Ejaculate volume (mL)	0.77 ± 0.12	0.84 ± 0.22
Concentration ($\times 10^9$ cells/mL)	4.14 ± 1.44	3.60 ± 0.81
Total sperm per ejaculate ($\times 10^9$ cells)	3.24 ± 1.45	3.12 ± 1.29
Massal motility (0–5)	4.15 ± 0.63	3.94 ± 0.99
Progressive individual motility (%)	81.00 ± 6.58	84.67 ± 4.42
Major defect (%)	15.55 ± 3.36	12.49 ± 3.15
Minor defect (%)	1.00 ± 0.53	2.13 ± 1.17
Total defect (%)	16.55 ± 3.46	14.61 ± 3.33

goats by solid-phase sandwich ELISA using high-sensitivity kit (<1 pg/mL; RayBiotech, Norcross, GA, USA). Three serum samples were collected from all animals throughout the experiment, namely, in their first day of life, at 1 month old (during) and at 10 months old (after suckling). After collection, samples were subsequently frozen until hormone assay. All procedures were performed according to the manufacturer instructions. The optical density was measured at 450 nm in a plate reader (Multiskan FC, Thermo Scientific, Helsinki, Finland).

Statistical analyses

Data are presented as mean ± standard deviation (s.d.) and were compared using the Graph Pad Instat 3.6 software (GraphPad Software, San Diego, CA, USA) at a confidence level of 95%. BW at birth, mean BW gain before weaning, BW at weaning, mean BW gain after weaning, BW at puberty, age at puberty, ejaculate volume, concentration, total sperm per ejaculate, massal motility, progressive individual motility, major defects, minor defects, total defects and serum biochemistry analysis of T and NT were analysed by Student's *t*-test. Due to lack of normality of the WBC data, Mann–Whitney–Wilcoxon test was used.

Results

Bodyweight development

Over 2 years of the trial, no significant (*P* > 0.05) differences were detected between T and NT animals regarding their BW

development (Table 1). In addition, numerically similar values were observed in females and males respectively, for BW at birth (T: 2.62 ± 0.38 kg; NT: 2.20 ± 0.00 kg; and T: 2.43 ± 0.11 kg; NT: 2.38 ± 0.16 kg), mean BW gain (kg/week) before weaning (T: 0.85 ± 0.10 kg; NT: 0.74 ± 0.24 kg; and T: 0.88 ± 0.17 kg; NT: 0.86 ± 0.25 kg), mean BW gain (kg/week) after weaning (T: 0.28 ± 0.46 kg; NT: 0.27 ± 0.50 kg; and T: 0.19 ± 0.43 kg; NT: 0.18 ± 0.29 kg), BW at weaning (T: 13.67 ± 1.19 kg; NT: 11.80 kg; and T: 13.80 ± 1.34 kg; NT: 13.55 ± 2.55 kg), and BW at puberty goats (T: 18.50 ± 3.93 kg; NT: 17.69 kg; and

T: 16.70 ± 1.34 kg; NT: 13.97 ± 1.92 kg). The comparison between males and females were not statistically analysed due to a small group size (<3 animals).

Puberty, physical and morphological characteristics of semen

The age at puberty (months) was similar ($P > 0.05$) between T (5.13 ± 1.00) and NT (5.06 ± 1.20). Regarding the gender, females and males respectively, were observed to be pubertal at
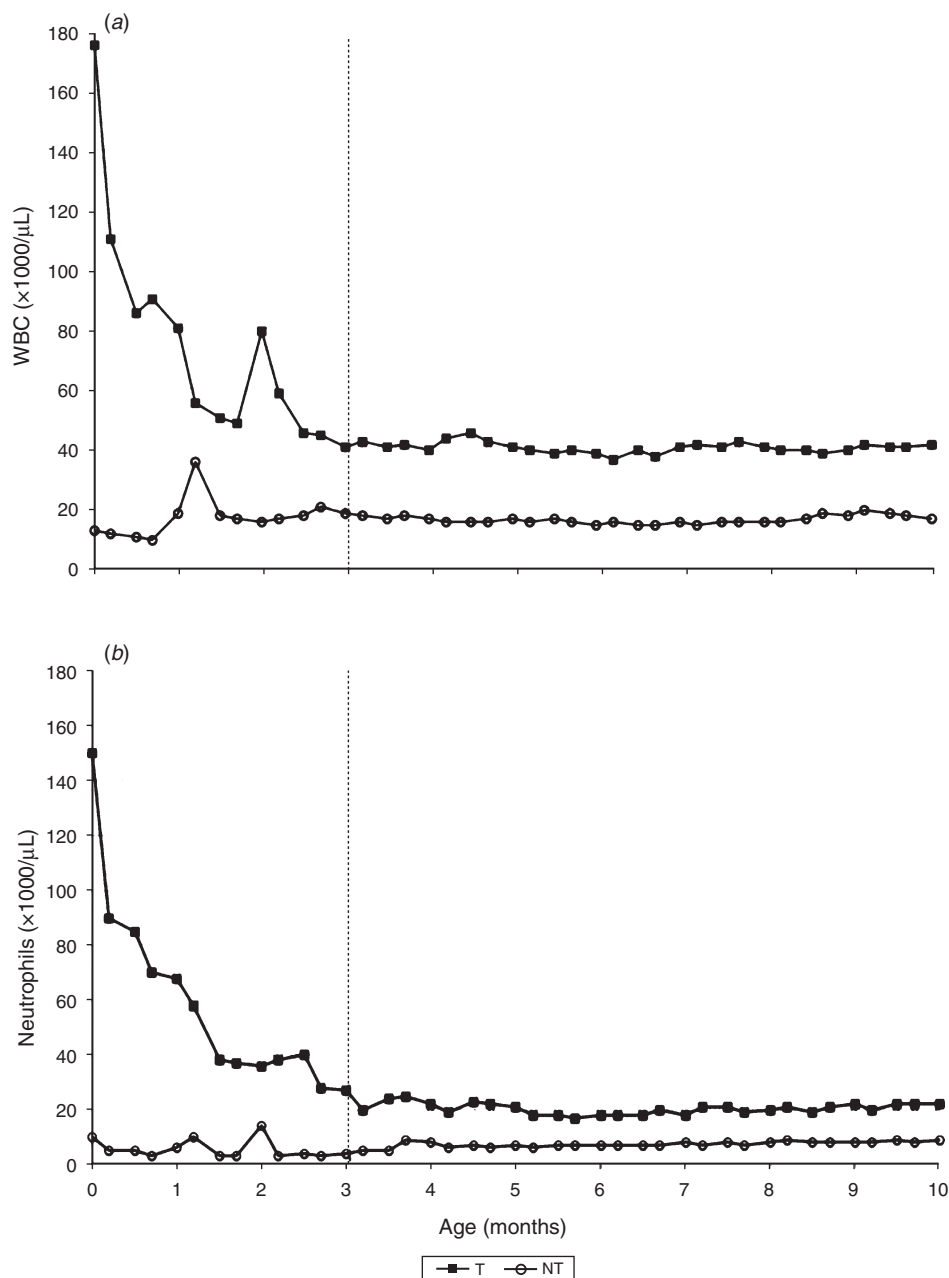

Fig. 1. Leukocyte profile of F1-generation hG-CSF-transgenic (T; *n* = 5) and non-transgenic (NT; *n* = 4) goats during (until dotted line) and after (after dotted line) breastfeeding. (*a*) White blood cell (WBC) and (*b*) neutrophil count.

5.30 ± 1.40 (T) and 6.77 (NT), and 4.90 ± 0.00 (T) and 4.50 ± 0.49 (NT) months. Interestingly, regardless to the presence of transgene, females were detected to be in puberty at 5.66 ± 1.37 months, whereas males were in puberty at 4.67 ± 0.40.

No significant ($P > 0.05$) differences between T and NT males were observed in any of the semen parameters evaluated (Table 2). Regardless of the presence of transgene, the overall means of the following variables were as follows: ejaculate volume 0.8 ± 0.12 mL, concentration $3.81 ± 0.99 × 10^9$ cells/mL, total sperm per ejaculate $3.17 ± 1.23 × 10^9$ cells/mL, massal motility 4.0 ± 0.4, progressive individual motility 83.2 ± 1.6%, major defects 13.71 ± 2.49%, minor defects 1.68 ± 0.40%, and total defects 15.39 ± 2.88%.

Hematological and serum biochemistry evaluations

Total WBC count for T and NT goats throughout the experimental period is shown in Fig. 1*a*. Significant ($P < 0.05$) differences in the mean WBC were detected between T and NT in their first day of life, and during and after suckling (Table 3). Although other cell counts were occasionally higher in T goats, differential counts showed that this difference was mainly due to

an increased number of neutrophils, which represented 84.6%, 67.2% and 56.8% of total WBC respectively, in the three time periods (Fig. 1*b*). The serum biochemical parameters of T and NT goats throughout the experimental period are presented (Table 4). In comparison with NT goats, no alterations occurred in serum concentrations of glucose, urea, creatinine, AST and ALT in T goats.

Human G-CSF analysis in serum

In all nine goats (T and NT), hG-CSF protein was not detected in any serum blood samples, in their first day of life, or during or after suckling.

Discussion

In the present study, several parameters associated with growth and reproduction of F1-generation transgenic goats for hG-CSF were evaluated to assess the performance of these lines (10M and 12F) when compared with their NT siblings. As the hG-CSF transgenic line was generated by pronuclear microinjection (Freitas *et al*. 2012), randomly integrated foreign DNA would be of concern, since it may increase the risk of disruption of

Table 3.   Leukocyte count (mean ± s.d.) in peripheral blood of F1-generation hG-CSF transgenic (T) and non-transgenic (NT) goats

Number of animals is shown in parentheses. Within a column, values with different letters represent a significant difference (at $P = 0.05$). During breastfeeding, the average was obtained from the second day of life up to 3 months old; after breastfeeding, average was obtained from 3 to 10 months old; normal range, reference values reported by Pugh (2002); WBC, total white blood cell count

Life period	Group	Cells ($×10^3$/μL)					
		WBC	Neutrophils	Lymphocytes	Eosinophils	Basophils	Monocytes
First day of life	T (5)	174.6 ± 14.7a	149.7 ± 19.3a	16.9 ± 5.8a	0.7 ± 0.8a	0.0 ± 0.0a	2.8 ± 1.0a
	NT (4)	15.0 ± 4.0b	12.6 ± 5.0b	3.2 ± 3.3b	0.1 ± 0.2a	0.0 ± 0.0a	0.2 ± 0.3b
During breastfeeding	T (5)	66.8 ± 21.1a	44.9 ± 20.0a	20.0 ± 6.8a	0.8 ± 0.7a	0.0 ± 0.0a	0.7 ± 0.5a
	NT (4)	17.0 ± 4.6b	7.2 ± 3.2b	12.2 ± 6.9a	0.3 ± 0.3a	0.0 ± 0.0a	0.1 ± 0.1a
After breastfeeding	T (5)	36.6 ± 4.0a	20.8 ± 3.4a	13.5 ± 2.3a	1.9 ± 0.9a	0.0 ± 0.0a	0.4 ± 0.2a
	NT (4)	15.5 ± 2.2b	6.2 ± 1.0b	8.1 ± 1.6a	0.5 ± 0.2a	0.0 ± 0.0a	0.1 ± 0.1a
Normal range		4.0–13.0	1.2–7.2	2.0–9.0	0.05–0.65	0–0.12	0–0.55

Table 4.   Serum biochemistry of F1-generation hG-CSF-transgenic (T) and non-transgenic (NT) goats

Number of animals is given in parentheses. AST, aspartate aminotransferase; ALT, alanine aminotransferase

Life period	Group	Biochemical dosages (mean ± s.d.)				
		Glucose (mg/dL)[A]	Urea (mg/dL)[B]	Creatinine (mg/dL)[A]	ALT (IU/L)[A]	AST (IU/L)[A]
First day of life	T (5)	91.02 ± 7.51	39.40 ± 6.55	0.95 ± 0.11	12.37 ± 5.42	61.14 ± 3.70
	NT (4)	100.37 ± 17.29	33.55 ± 3.52	0.91 ± 0.10	15.00 ± 6.75	63.04 ± 12.62
During breastfeeding[A,C]	T (5)	56.57 ± 5.32	36.80 ± 2.91	1.02 ± 0.04	19.80 ± 3.65	62.68 ± 4.00
	NT (4)	57.84 ± 3.53	36.61 ± 3.74	1.09 ± 0.17	18.63 ± 2.33	60.05 ± 4.30
After breastfeeding[B,D]	T (5)	50.96 ± 4.24	37.89 ± 0.74	1.06 ± 0.22	15.60 ± 4.36	57.80 ± 8.66
	NT (4)	52.84 ± 4.32	30.92 ± 1.51	1.10 ± 0.07	18.50 ± 7.23	66.25 ± 12.17
	Normal range	48.2–76.0	21.4–42.8	0.7–1.5	15.3–52.3	66.0–230.0

[A]Reference value reported by Boyd (1984).
[B]Reference value reported by Kaneko *et al*. (1997).
[C]Average obtained from the second day of life up to 3 months old.
[D]Average obtained from 3 to 10 months old.

endogenous genes (Jackson *et al*. 2010) and disturb normal physiological processes. In this context, it has been suggested that three aspects of the welfare of a transgenic line should be evaluated, including the effects of insertional mutagenesis, transgene expression and, if applicable, effects of *in vitro* reproduction technologies if somatic-cell nuclear-transfer techniques were used to generate the transgenic line (Van Reenen 2009).

None of the growth and reproductive traits was affected by the presence of the transgene. No significant differences between T and NT were observed in the BW at birth, mean BW gain before weaning, mean BW gain after weaning, BW at weaning and BW at puberty, suggesting that the site of integration of the transgene did not appear to interrupt an endogenous gene for growth *in utero* and absorption of nutrients after birth. During all experimental periods, the mean BW gains observed in the present study were 0.06 and 0.05 kg per day for T and NT respectively, all within the range of 0.02–0.3 kg per day reported in the literature for goats (National Research Council 2007).

The age at puberty observed for males and females, for both T and NT, were within the range reported in literature for caprine species (3–7 months of age; Jackson *et al*. 2010) and the onset of puberty initiated when expected considering their BW. Additionally, semen physical (ejaculate volume, concentration, total sperm per ejaculate, massal motility, progressive individual motility) and morphological (major, minor, total defects) characteristics were similar between both experimental groups. Values observed for these parameters are within the normal range for goats, namely, volume of 0.1–1.5 mL, concentration of 2–6 billion sperm per mL of ejaculate, progressive individual motility of 82–93% and normal morphology of 80–95% (Chandler *et al*. 1988; Nur *et al*. 2005). Overall, the presence of the hG-CSF transgene in the genome of these animals does not appear to interfere on their normal reproductive parameters. This result can be attributed to insertion site and heterozygosity of animals used in the present study. The heterozygous or homozygous status for the transgene has been shown to correlate with gene-expression levels and rates of the transmission of the transgene (Tesson *et al*. 2002) that could compromise the reproductive health of these animals.

Human G-CSF regulates the proliferation, differentiation and maturation of haematopoietic cells (Crea *et al*. 2009). This molecule exerts biological functions through specific receptors expressed on the surface of target cells (Richt *et al*. 2007), stimulating proliferation and differentiation of the neutrophil. During the beginning (acute) phase of inflammation, particularly as a result of bacterial infection, environmental exposure (Jacobs *et al*. 2010) and some cancers (Waugh and Wilson 2008; De Larco *et al*. 2004), neutrophils are one of the first-responders among inflammatory cells to migrate towards the site of inflammation. We observed, in the present study, an increase of 11.6-, 4.5- and 2.4-fold in the WBC count of T compared with NT goats in their first day of life, during and after suckling respectively. This result suggests that G-CSF, although a human protein, is able to affect goat haematopoietic stem cells, stimulating their proliferation and differentiation, mainly for neutrophils. It is important to highlight that we did not detect the presence of this cytokine in the serum in any T goats, at different life periods of evaluation.

Additionally, no ectopic hG-CSF expression was detected in the studied tissues of F1 transgenic males (Batista *et al*. 2014; Melo *et al*. 2015). It is noteworthy that neither chronic nor acute kidney disease was detected, as demonstrated by normal concentrations of urea (primary metabolite derived from dietary protein and tissue protein turnover) and creatinine (product of muscle catabolism). Likewise, indicators of liver function (ALT and AST) suggested normal hepatic functioning, as well as normal glycemic level. Finally, this information can provide support contributing to the knowledge required to make science-based regulatory decisions regarding the use of transgenic animals. Therefore, these results suggest a greater ability to respond to infection by these transgenic animals than by non-transgenic animals.

Conclusions

Despite the increased WBC count due to neutrophilia, first-generation hG-CSF-transgenic male and female goats remained clinically healthy throughout the experimental period, and remain so until now. Their BW development was considered normal both prenatally and during their first months of age. Moreover, all the reproductive characteristics evaluated, such as age at puberty and semen physiological and morphological characteristics, were not adversely affected by the insertion of the transgene. Therefore, it is possible to assume that all animals are normal and had no deleterious effects on either growth or reproductive parameters by the presence of the transgene or as a consequence of leukocyte profile alteration.

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