

# SUCCESSFUL LONG-TERM PRE-PUBERTAL IMMUNOCASTRATION OF PUREBRED IBERIAN GILTS REARED IN EXTENSIVE SYSTEMS

Francisco I. HERNÁNDEZ-GARCÍA <sup>1,2</sup>, Juan L. DUARTE <sup>1</sup>, Miguel A. PÉREZ <sup>1</sup>,  
Cristina RABOSO <sup>1</sup>, Ana I. del ROSARIO <sup>1</sup>, Mercedes IZQUIERDO <sup>1</sup>

## ABSTRACT

Iberian pigs are raised in Spain in extensive, free-ranging systems in which males and females are gonadectomized to avoid several management problems. However, the new animal welfare regulations restrict female surgical sterilization. The objective of the present study was to develop a feasible immunocastration protocol to prevent oestrus cyclicity from the pre-pubertal period until slaughter, typically performed in extensively reared Iberian pigs that are older and have higher weight. This early immunocastration protocol, with no need for separation of sexes before immunization, would simplify herd management in extensive systems. Six Iberian gilts (Treated) were immunized with Improvac<sup>®</sup> (Zoetis-Pfizer) at 19, 23 and 39 weeks of age and were slaughtered at 68 weeks of age. Other 6 Iberian gilts were used as Controls. Serum progesterone levels (from monthly and weekly samplings) and post-mortem utero-ovarian measurements indicated ovarian and oestrus cyclicity in Control gilts and ovarian quiescence in Treated gilts, whose reproductive tracts remained immature until slaughter. In conclusion, this 3-dose, pre-pubertal immunization protocol offers a highly effective alternative to ovariectomy of Iberian pigs for the prevention of estrus in extensive systems.

**Key words:** Iberian pig / reproduction / GnRH inhibition / Improvac<sup>®</sup> / female / puberty

## 1 INTRODUCTION

Due to the new regulations on animal welfare, the Iberian pig Sector must find an alternative to surgical gonadectomy of males and, most urgently, of females. In 2009, an anti-GnRH vaccine (Improvac<sup>®</sup>; Pfizer) was licensed in the E.U. for use in male pigs in a two-dose protocol. According to producer, its effect is reversible, but sufficiently prolonged to inhibit boar taint accumulation during the growing-finishing period in white pig breeds (Dunshea *et al.*, 2001), which is much shorter than for Iberian pigs, especially if the latter are reared in extensive systems. On the other hand, the GnRH inhibition could also be used to prevent estrus and ovulation in females. In fact, there are experimental data (Oliver *et al.*, 2003) showing this effect in Improvac-treated female white pigs during their finishing phase, in a time protocol similar to the one used in males. However, the cured ham industry

needs heavier and older animals of white-based breeds, and the cured Iberian ham industry uses even heavier and much older pigs, due to the longer growing-finishing periods and the specific curing techniques (Peinado *et al.*, 2008). Therefore, the immunization protocols for immunocastration of Iberian males and females must be modified, specially to avoid aggressive behavior and unwanted oestrus activity or pregnancies during the prolonged free-ranging periods, in which both sexes are usually kept together to complete the finishing phase in the extensive systems, either in a regular (concentrate-based) extensive fattening or in the so called *montanera* period for the acorn-fed Iberian pigs. At the start of *montanera*, the pigs should have a body weight of 100–115 kg, and, usually after 2 to 4 months, the finished pigs exit the *montanera* and are slaughtered reaching around 160 kg of body weight. In addition, for the high-grade quality of acorn-fed Iberian pig products (provided with cer-

<sup>1</sup> Agricultural Research Center of Extremadura, 06187-Guadajira, Badajoz, Spain

<sup>2</sup> Corresponding author, e-mail: francisco.hernandez@juntaextremadura.net

tification of origin), the legal regulations give a narrow range for these timing and weights. Nevertheless, regular extensive fattening tends to finish at a similar age and weight. Therefore, the objective of the present study was to develop a feasible immunocastration protocol to prevent oestrus cyclicity before puberty, so that herd management in extensive systems is easier, with no need for sex separation, and also to maintain its effect for a period long enough for extensively-reared Iberian females during the prolonged growing and finishing phases.

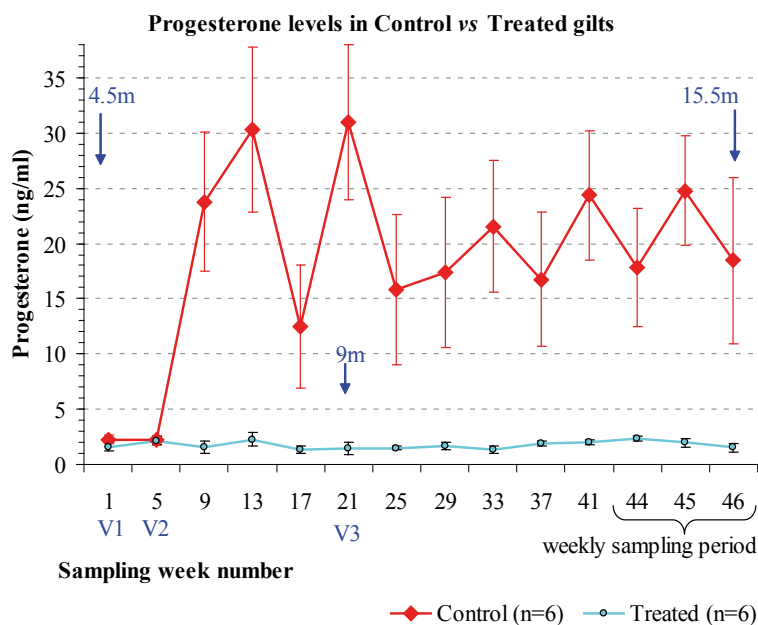
## 2 MATERIALS AND METHODS

Twelve purebred Iberian gilts with a mean weight (at 23 weeks of age) of 52 kg (range 46–50 kg) were used in this study. Animals were housed in a large outdoor pen and commercial pig growing and finishing concentrates were fed at a progressive rate from 1 to 1.8 kg/head/day from weaning to 105 kg of body weight or until 12 months of age (whichever came first). Afterwards, during the final finishing period, the ration was increased to 4 kg/head/day. Treated gilts (pre-pubertally immunocastrated females;  $n = 6$ ) were injected 2 mL of Improvac® (Zoetis-Pfizer; at commercial formulation, with 375 µg/dose of GnRH analogue-protein conjugate) subcutaneously behind the ear. The remaining gilts (intact females;  $n = 6$ ) were used as Controls. A 3-dose immunization protocol with Improvac® (see Fig. 1 for the timing) was carried out as follows: the first vaccination was per-

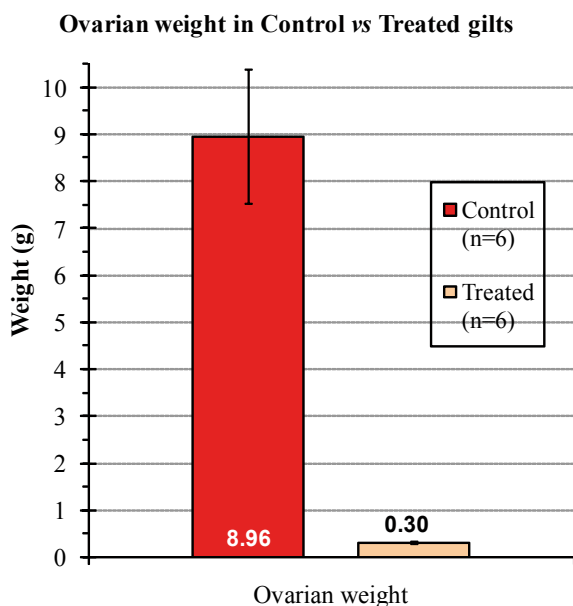
formed at 19 weeks of age (in June 2011), the second dose at 23 weeks of age (at least 4 weeks after the first, priming dose, as indicated by the manufacturer) and the third dose was given 16 weeks after the second one, at 39 weeks of age. The second vaccination was intended to take place a short time before the end of the pre-pubertal period, to avoid unwanted oestrus cyclicity. González-Añover *et al.* (2010) showed that purebred Iberian gilts reach puberty at an earlier age than white breed pigs, with 72% of cycling Iberian females (versus 15%) at 6 months of age (180 days). Gilts were slaughtered in May 2012 with a mean body weight of 153 kg (range 134–173 kg) at 68 weeks of age, 32 weeks after the last vaccination.

A blood sample was taken from each gilt for the analysis of serum concentrations of progesterone, at the time of Improvac® administration, thereafter every 4 weeks until a 3-week pre-slaughter period, in which blood sampling was performed weekly to assess oestrus cyclicity. Progesterone was quantified using a commercially available ELISA kit (Enzo Life Sciences).

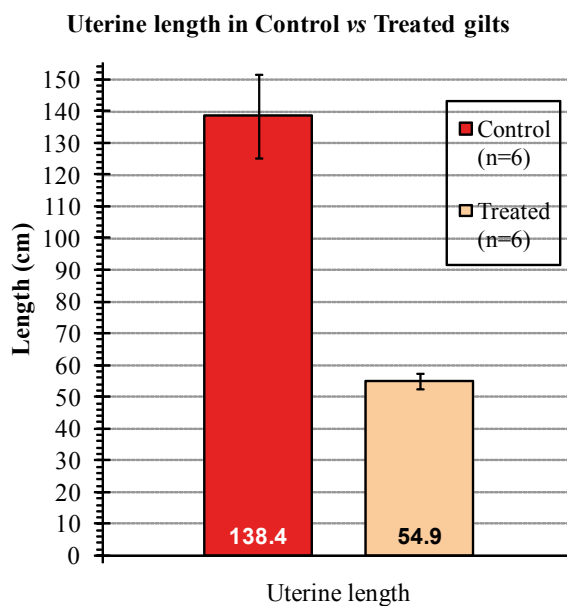
At *post-mortem* examination, the weight and length of the uterine horns (right and left together) were determined after removal of the uterine broad ligament. Also, the ovaries were weighed and scored in relation to the number and size of the follicles and *corpora lutea*, as follows: 0 (no visible follicles); 1 (3–4 mm; immature follicles); 2 (8–11mm; mature follicles); 3 (*corpora lutea* and/or *corpora albicantia*, and 3–11 mm follicles; cyclic ovary).



**Figure 1:** Serum progesterone levels (means  $\pm$  SE) in Control vs. Treated (pre-pubertally immunocastrated) Iberian gilts. Age at samplings (from June 1 2011 to May 9 2012) is indicated by vertical arrows (m – months); V – vaccination number (3 Improvac doses)



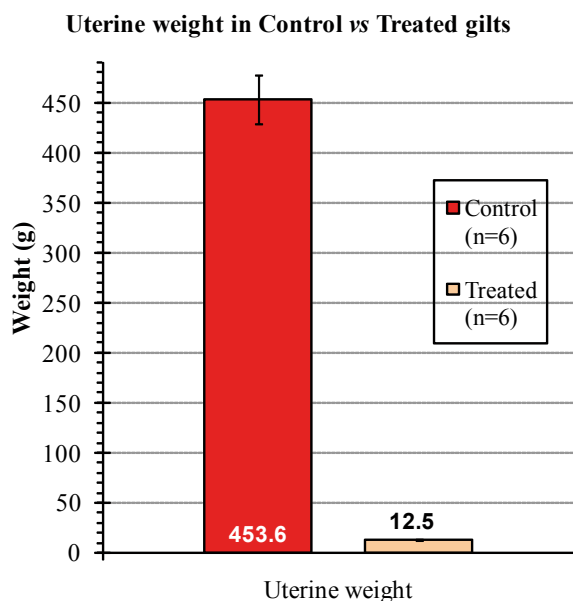
**Figure 2:** Post-mortem ovarian weight (bilateral mean) in Control vs. Treated (pre-pubertally immunocastrated) Iberian gilts at 16 months of age. Data are means  $\pm$  SE.



**Figure 4:** Post-mortem, bilateral uterine horn length in Control vs. Treated (pre-pubertally immunocastrated) Iberian gilts at 16 months of age. Data are means  $\pm$  SE.

### 3 RESULTS AND DISCUSSION

From the day of the 2<sup>nd</sup> vaccination onwards (Fig. 1), progesterone levels for Treated gilts were significantly lower in comparison with Control gilts. For the Control gilts, the acute hormonal change from the 2<sup>nd</sup> vaccina-



**Figure 3:** Post-mortem, bilateral uterine horn weight in Control vs. Treated (pre-pubertally immunocastrated) Iberian gilts at 16 months of age. Data are means  $\pm$  SE.

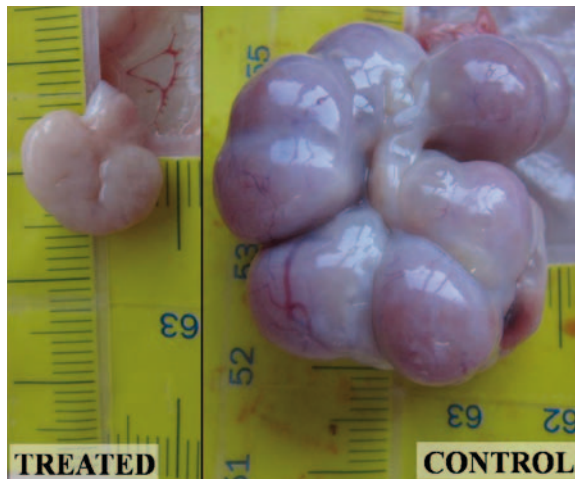
tion day until the next sampling week showed the post-pubertal progesterone rise (Fig. 1), whereas Treated gilts did not seem to reach puberty. Moreover, the final weekly progesterone profiles, depicting mean values (Fig. 1) or individual data (not shown), indicate ovarian cyclicity for Control gilts and ovarian quiescence for Treated gilts.

The inhibition of puberty was more evident after examining the reproductive tract of Treated gilts at slaughter, as their uteri and ovaries were in a pre-pubertal, infantile state (Fig. 2–5). It is plausible that reproductive organs did not undergo an infantilization process, due to the fact that the 2<sup>nd</sup> – effective vaccination was given pre-pubertally and, moreover, that progesterone levels were close to basal at all sampling occasions (the lower detection limit was 0.34 ng/mL; individual data not shown), and they only reached 4 ng/mL in 2 occasions (4.9 and 4.1 ng/mL for one treated gilt at 32 and 40 weeks of age, respectively).

At slaughter (32 weeks after the 3<sup>rd</sup> vaccination), the mean ovarian weight was significantly, 30-fold greater, in Control than Treated gilts (Fig. 2).

Ovarian weight was significantly, 30-fold greater in Control than Treated gilts (Fig. 2). Similarly, the uterine horns of Control gilts were significantly heavier (36-fold; Fig. 3) and significantly longer (2.5 fold; Fig. 4) than those of Treated gilts. Moreover, all Treated gilts had completely immature ovaries, of very small size, smooth surface, and lacking visible follicles (score 0). In contrast, all Control gilts had mature, cyclic ovaries (score 3; Fig. 5).

Presented results indicate ovarian activity and



**Figure 5:** Representative ovaries (on same scale) of Treated (pre-pubertally immunocastrated) and Control Iberian gilts at 16 months of age. All Treated gilts had immature ovaries, of very small size, smooth surface, and lacking visible follicles (score 0). All Control gilts had mature, cyclic ovaries (score 3), in this case in luteal phase.

normal oestrus cyclicity in Control gilts and ovarian quiescence in Treated gilts, whose reproductive tracts remained immature until slaughter. Therefore, immunization protocol with vaccinations applied at 19, 23 and 39 weeks of age has proven to be effective until slaughter at 68 weeks (16 months) of age, which fits well with the time frame of extensive Iberian pig operations.

In conclusion, presented results show that the 3-dose, pre-pubertal immunization protocol developed

in the present study offers a highly effective alternative to surgical sterilization of female Iberian pigs for the prevention of estrus in extensive rearing systems.

#### 4 ACKNOWLEDGEMENTS

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