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A. González-Bulnes*, T. Encinas, E. Gómez-Izquierdo***, P. González-Añover****, P. Pallarés*, M.L. Pérez-Solana*, R. Sánchez-Sánchez* and L. Torres-Rovira***

*Dpto. de Reproducción Animal, INIA, Avda. Puerta de Hierro s/n. 28040 Madrid (Spain)
**Dpto. de Toxicologia y Farmacologia, Facultad de Veterinaria, UCM. Madrid (Spain)
***Centro de Pruebas de Porcino, ITACyL, Segovia (Spain)
****NUTEGA, Coslada, Madrid (Spain)

Abstract. The influence of ovulation rate and embryo viability on the lower prolificacy of Iberian sows when compared to modern commercial breeds was firstly assessed in 78 non-pregnant cycling Retinto females showing a mean number of 12.7±0.2 corpora lutea (CLs). However, 66.1% of the females had ovulation rates under the mean (9.9±0.2 CLs); the remaining sows had 16.9±0.4 CLs (P<0.05). Such distribution was confirmed in 23 pregnant Retinto sows having 14.8±2.4 CLs; 69.5% of the females had lower ovulation rates (11.8±1.0 CLs), the remaining sows had 18.2±3.9 CLs (P<0.05). However, sows having high ovulatory rates showed, at Day 35 of pregnancy, a high incidence of CLs regression and embryo losses. Such effects were not found in females with low ovulation rates and, thus, number of viable embryos was finally similar in both groups (8.2±1.0 and 8.4±1.0). These results, indicating that prolificacy of Iberian sows is more influenced by embryo losses in the first third of pregnancy than by ovulation rate, was confirmed by studying 18 females of a prolific Retinto x Torbiscal strain. Mean ovulation rate was 21.3±0.5 CLs, but embryo losses reached a mean of 46.6%; the incidence of embryo mortality correlated linearly with ovulation rate (r=0.819, P<0.01).

Keywords. Embryo mortality – Ovulation – Pregnancy – Swine.

Le rôle relatif des taux d'ovulation et des pertes d'embryons sur la prolificité des truies Ibériques

Résumé. *L'influence des taux d'ovulation et de la viabilité des embryons sur une prolificité inférieure des truies Ibériques par rapport aux races commerciales modernes a été tout d'abord évaluée chez 78 femelles de Retinto non gestantes présentant un nombre moyen de 12,7 ± 0,2 corps jaunes (CJs). Toutefois, 66,1% des femelles avaient des taux d'ovulation sous la moyenne (9,9 ± 0,2 CJs); les autres truies avaient un taux de 16,9 ± 0,4 CJs (P <0,05). Cette distribution a été confirmée chez 23 truies Retinto gestantes ayant un taux de 14,8 ± 2,4 CJs ; 69,5% des femelles avaient des taux inférieurs d'ovulation (11,8 ± 1,0 CJs) et les truies restantes présentaient un taux de 18,2 ± 3,9 CJs (P<0,05). Toutefois, les truies qui avaient des taux d'ovulation élevés ont montré au jour 35 de la gestation une forte incidence de régression de CJs et de pertes d'embryons. Ces effets n'ont pas été rencontrés chez les femelles ayant des taux d'ovulation faibles et, par conséquent, le nombre d'embryons viables a été finalement similaire dans les deux groupes (8,2 ± 1,0 et 8,4 ± 1,0). Ces résultats, indiquant que la prolificité des truies Ibériques est plus influencée par les pertes d'embryons dans le premier tiers de la gestation que par le taux d'ovulation, ont été confirmés par l'étude de 18 femelles d'une race prolifique Retinto x Torbiscal. Le taux d'ovulation moyen était de 21,3 ± 0,5 CLS, mais les pertes d'embryons ont atteint une moyenne de 46,6%; l'incidence de la mortalité des embryons est en corrélation linéaire avec le taux d'ovulation (r = 0,819, P <0,01).*

Mots-clés. *Mortalité embryonnaire – Ovulation – Gestation – Porc.*

I – Introduction

The Iberian pig, like other Mediterranean swine breeds, is characterized by a clear predisposition to fat accumulation under its skin and among the muscular fibres (Nieto *et al.* 2002). This pattern of fat storage has been found not only in Iberian pigs but also in other animal species and even in humans, being named as *thrifty genotype* (Neel, 1962). The *thrifty genotype* is an adaptive mechanism to the environment, allowing accommodation to seasonal cycles of feasting and famine. The ability to store excess fat enables survival during periods of scarcity, but individuals with *thrifty genotype* become obese when food is in excess.

The abundance of fat in Iberian pigs causes an increased secretion of leptin when compared to lean swine breeds (Fernandez-Figares *et al.,* 2007), which has been also described for the Mangalica pig (Brüssow *et al.,* 2008). Leptin is the hormone produced in the adipose tissue for regulating appetite and food intake (Zhang *et al.,* 1994, Houseknecht *et al.,* 1998). The hormone was not identified until 1994 when cloning of the mouse obese gene was achieved (Zhang *et al.,* 1994). The obese mouse (ob/ob mouse), discovered in 1949 (Ingalls *et al.,* 1950), has deficiencies in leptin secretion and is characterized as being grossly overweight, due to high food consumption and scarce physical activity, and, additionally, was established as hyperglycemic, hyperlipidemic, and hyperinsulinemic. Consequently, these mice have been extensively used as a model for obesity.

However, in spite of high leptin secretion, it has been found that the Iberian pig has a gene polymorphism of the leptin receptors (LEPR) with effects on food intake, body weight and fat deposition (Ovilo *et al.,* 2005; Muñoz *et al.,* 2009); as a consequence, Iberian LEPR alleles increase insatiability and obesity. The same syndrome has been also described in human medicine and named as *leptin resistance*; some obese individuals have elevated leptin levels but fail in suppressing feeding (Martin *et al.,* 2008; Myers *et al.,* 2008) due to LEPR polymorphisms (Mizuta *et al.,* 2008).

Obesity and obesity-associated hyperleptinaemia by *leptin resistance* in humans have been linked to reproductive disorders (Metwally *et al.,* 2008; Brewer *et al.,* 2010). Leptin also has a key role in reproduction, acting through its receptors in hypothalamus, pituitary, ovary and endometrium (Cioffi *et al.,* 1997, Yu *et al.,* 1997, Gonzalez *et al.,* 2000, Duggal *et al.,* 2002, Watanobe 2002, Welt *et al.,* 2004). In fact, the obese syndrome also affects reproductive function (Lindström, 2007); a consequence of which both male and female ob/ob mice are infertile. Infertility is a direct consequence of leptin deficiency; thus, the administration of leptin restores weight, metabolic function and fertility (Barash *et al.,* 1996; Pallares *et al.,* 2010). Other strain of obese mice, in this case with monogenic deficiencies in LEPR ($Lepr^{db/db}$), is also infertile (Tartaglia *et al.*, 1995). In women, hyperleptinaemia has been related to menses irregularities, chronic oligo-anovulation and infertility; may be through a direct impairment of ovarian function (Pasquali and Gambineri 2006, Pasquali *et al.,* 2007), leading to alterations of granulosa cell function and follicle development (Fedorcsák *et al.,* 2000, Pasquali *et al.,* 2006), or may be through alterations in early-pregnancy and implantation (Bellver *et al.,* 2007).

The Iberian pig is also characterized by a lower reproductive efficiency, specifically a lower prolificacy (López-Bote, 1998), than modern commercial breeds; the same has been found in the Mangalica breed (Rátky *et al.,* 2005). Prolificacy in swine, as in other multiparous species, may depend on ovulation rate and/or embryo losses during pregnancy. Thus, the objective of this study was to characterize, for the Iberian breed, the consistency of ovulation rate and, thereafter, the incidence of embryo losses between Days 21 and 40 of pregnancy; a critical period that comprises from achievement of trophoblast attachment and implantation to completion of the transition from late embryo to early foetal stage (Ashworth *et al.,* 2006; Whittemore and Kyriazakis, 2006). For evaluating effects of the strain, pregnant females from purebred Retinto and a recombinant congenic strain with 75% Retinto and 25% Torbiscal (commonly used for breeding in practice) were compared.

II – Materials and methods

A total of 119 nulliparous Iberian sows, with a mean age of around 40 weeks and no previous evidence of health problems and adequate pathogen-monitoring reports, were used; all of them were genotyped for LEPR gene polymorphisms, as previously described (Ovilo *et al.,* 2005). Animals were housed indoors, in passively ventilated pens with concrete slatted floors, at either the Centro de Pruebas de Porcino (CPP, ITACyL, Hontalbilla, Segovia, Spain) or the INIA (Madrid, Spain). These facilities meet the local, national and European requirements for Scientific Procedure Establishments.

Assessment of ovulation rate was performed in a first experiment, by the observation of luteal structures in spontaneous non-induced oestrous cycles from 78 non-pregnant Retinto females reared at the CPP facilities from weaning. Ovaries were obtained at the slaughterhouse and, immediately after removal of the genital tracts, ovulation rate was determined by assessing the presence and number of luteal structures (*corpora haemorraghica*, *lutea* and *albicans*; Fig. 1).

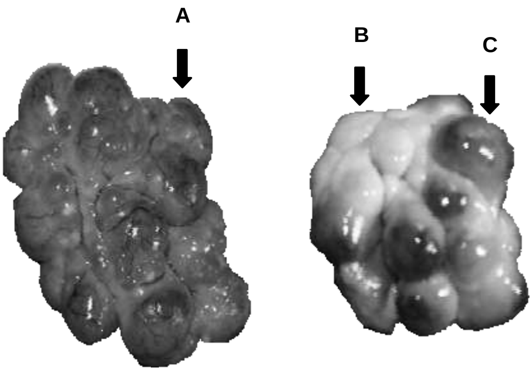

Fig. 1. Ex vivo image of corpora lutea (A), albicans (B) and haemorraghica (C).

Assessment of the relative roles of ovulation rate and embryo losses on prolificacy was performed in two consecutive experiments using 23 pregnant sows of Retinto strain and 18 pregnant Retinto x Torbiscal crossbreed females. These animals were treated, for cycle synchronization and breeding, with 20 mg of the progestagen altrenogest (Regumate®, Intervet International, Boxmeer, The Netherlands), daily for 18 consecutive days, by individually top-dressing over their morning feed; the treatment being initiated irrespective of the stage of the cycle. Oestrus detection was performed twice daily, from 24 h after progestagen removal; both inspection of the vulva for reddening and swelling (pro-oestrus) and control of the standing reflex (oestrus) in contact with a mature boar were performed. Sows were inseminated 12 and 24 hours after oestrus detection. Entire genital tracts were collected, between Days 21 (9 Retinto females) and 40 of pregnancy (14 Retinto and 18 Retinto x Torbiscal females), for evaluation of ovulation rate and characterization of conceptuses. Ovulatory sites in the ovaries were assessed for determining ovulation rate and evaluating morphologically normal and regressing corpora lutea. Thereafter, contents of the uterus were exposed and implantation sites and viable and non-viable conceptuses were recorded (Fig. 2) and compared to the number of corpora lutea.

For the statistical analyses, data from both pregnant and non-pregnant sow were summarized to characterize ovulation rate. Thereafter, data obtained were grouped according to genotype and day of gestation and the effects of these variables on number and characteristics of corpora lutea and conceptuses were tested by analysis of variance (ANOVA). Possible relationships between number and characteristics of corpora lutea and conceptuses were tested by Pearson correlation analysis and Spearman nonparametric correlation tests for non homogeneous variables. The parameter values were expressed as means ± SEM, and statistical significance was accepted for P< 0.05.

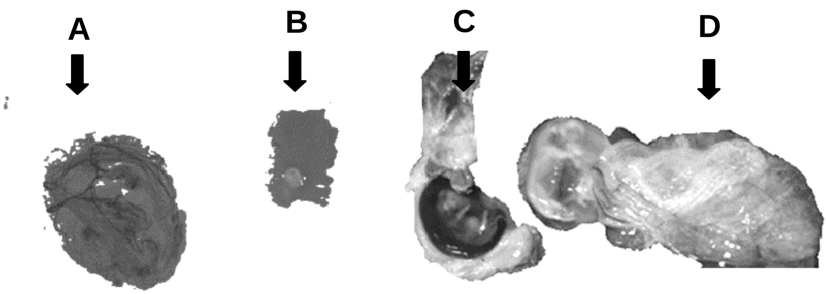

Fig. 2. ***Ex vivo*** **image of viable (A and D) and non-viable (C and B) embryos at Days 21 (A and B) and 35 (C and D) of pregnancy.**

III – Results

The assessment of the number of luteal structures in the non-pregnant cycling Retinto females showed a mean ovulation rate of 12.7±0.2. However, two different groups were found. Most of the females (66.1%) had ovulation rates under the mean (9.9±0.2), whilst the remaining sows (33.9%) had ovulation rates above the mean and significantly higher than in the first ones (16.9±0.4, P<0.05). Such distribution was confirmed when evaluating the ovulation rate in the 23 pregnant Retinto sows. The mean number of luteal structures was 14.8±2.4 CLs; 69.5% of the females had lower ovulation rates (11.8±1.0 CLs) and the remaining sows had 18.2±3.9 luteal structures (P<0.05). The ovulation rate in the Retinto x Torbiscal strain was higher than in both groups (pregnant and non pregnant) of Retinto females (21.3±1.4, P<0.005). The distribution of females with higher and lower ovulation rates was also different, since 50% of the animals had ovulation rates above and under the mean (25.4±1.4 *vs.* 17.1±1.1, respectively; P<0.01).

The incidence of embryo losses in the pregnant Retinto sows, and the comparison between 21 and 35 days of pregnancy, revealed that females with higher ovulatory rates showed a high incidence of luteal regression and embryo losses between Days 21 and 35 of pregnancy (around 50%) (Fig. 3). Thus, the number of viable embryos, at Day 35, was 8.2±1.0 in these females. On the other hand, such effects were not found in females with lower ovulation rates; in these sows the incidence of embryo losses was around 15% and the number of viable embryos at Day 35 was similar to the sows with higher ovulation rate (8.4±1.0).

In the Retinto x Torbiscal strain, embryo losses reached a mean of 46.6%, similarly to Retinto females with higher ovulation rate (Fig. 3). The incidence of embryo mortality correlated linearly with ovulation rate (r=0.819, P<0.01). Females with ovulation rate under the mean showed 31.2% of embryo losses; females with ovulation rate above the mean showed 55.9% of embryo losses. Thus, number of viable embryos in both groups were similar (11.8±0.7 *vs* 10.9±0.6), but higher than in Retinto females (P<0.01).

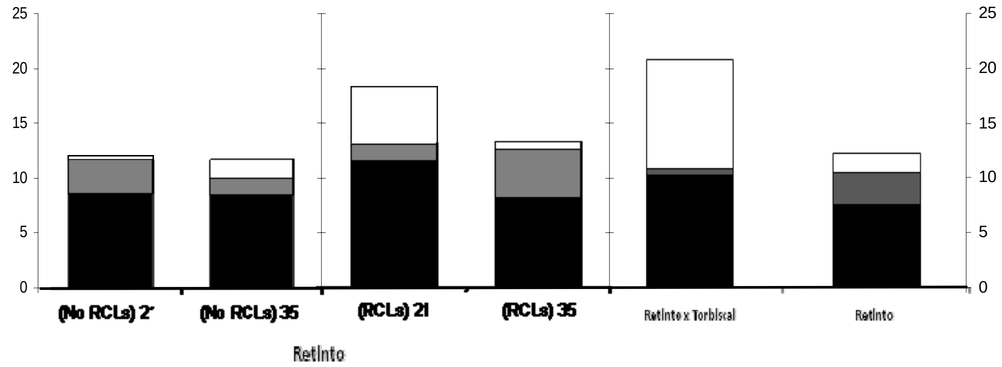

Fig. 3. Mean number of absent (white bar), degenerated (grey bar) and viable embryos (black bar) at Days 21 and 35 of pregnancy in Retinto sows with and without regressed corpora lutea (RCLs and NoRCLs) and comparison, at day 35-40 of pregnancy, between Retinto x Torbiscal and Retinto strains.

IV – Discussion

These results indicate that ovulation rate is not the main limiting factor for prolificacy of Iberian sows; prolificacy seems to be more influenced by embryo losses in the first third of pregnancy than by a lower ovulation rate.

When considering data about number of ovulations, the present results show a higher ovulation rate in the Retinto x Torbiscal than in the Retinto strain; which is in agreement with previous data obtained by farrowing observations (Suárez *et al.,* 2002a,b). Differences in the ovulation rate between the first and second replicates of Retinto may be related to the use of exogenous hormones in the second replicate. But, overall, our results suggest –besides a higher individual variability both in the Retinto purebred and in the Retinto x Torbiscal strain-two different "ovulatory behaviours". When evaluating the Retinto strain, most of the females (60%) had lower ovulation rates whilst some of them (40%) had high ovulation rates; the same was found in the Retinto x Torbiscal strain, although the distribution was around 50:50%. Females with higher ovulation rate also showed an intense luteal regression; thus, it seems that number of functional corpora lutea in pregnant Iberian sows was modulated either by a lower ovulation rate or by a lower quality of some of the corpora lutea in those females with higher ovulation rates.

Thereafter, prolificacy of pregnant sows was mainly hampered by differences in the number and viability of embryos. In swine, like in other mammals, prolificacy is determined by ovulation rate and/or embryo/foetal survival. The relative roles of ovulation rate and embryo survival has been extensively studied in the sow by using the Chinese Meishan pig, a very high prolific breed with average litter sizes 30 to 40% greater than European and American pigs (Bolet *et al.,* 1986; Haley and Lee 1993; Young 1993). These studies indicated, like in our study, that a higher number of corpora lutea in Meishan sows (Ashworth *et al.,* 1990; Haley and Lee, 1993, Anderson *et al.,* 1993; Christenson 1993), would not be enough to explain such higher prolificacy; issues related to a better embryo survival and developmental rate both at pre-implantational stages (Bazer *et al.,* 1988; Youngs *et al.,* 1993) and/or after implantation (Youngs *et al.,* 1993; Biensen *et al.,* 1999; Wilson *et al.,* 1999) would be definitive.

The present study, developed in Iberian sows, a different model with smaller litters than modern lean breeds, would be confirming the main role of embryo viability on swine prolificacy. Coincidentally, some authors have related deficiencies in reproductive outputs of obese females to alterations in early embryo development (Kawamura *et al.,* 2002; Fedorcsak and Storeng 2003), trophoblast function (Castellucci *et al.,* 2000) and endometrial receptivity (Alfer *et al.,* 2000; González *et al.,* 2000). The most recent studies in humans indicate an interrelationship between lower oocyte/embryo developmental competence and alterations in oviduct/uterine environment leading to deficiencies in early-pregnancy and implantation (Bellver *et al.,* 2007).

Implantation has been, and it is, widely studied in swine. Embryonic implantation is a crucial event in the establishment of pregnancy. Several factors have been reported to have roles in implantation and uterine receptivity (Giudice, 1994); within them, leptin. Leptin and LEPR (mRNA and protein) are expressed in the oviduct (Kawamura *et al.,* 2002; Craig *et al.,* 2005) and the endometrium (Gonzalez *et al.,* 2000; Kawamura *et al.,* 2002), suggesting possible involvement in endometrial receptivity for the developing embryo (Mitchell *et al.,* 2005). Moreover, the fact that LEPR are differentially regulated in implantation and inter-implantation sites suggests a regulatory role of the presence of an approaching embryo (Yoon *et al.,* 2005). Leptin and LEPR are known to be regulated by estradiol (Henson and Castracane, 2006) and embryonic estradiol is the signal for implantation in swine (Anderson *et al.,* 1993). Thus, it is possible to hypothesize that the different rate of protein secretion between implantation and inter-implantation sites described in the pig in the early 1990's would be regulated by estradiol and LEPR. We have to remind that Iberian pigs have leptin resistance and have a LEPR gene polymorphism disrupting processes of signal transduction; however, a possible relationship with implantation success may be tested by further studies.

V – Conclusions

These results suggest that prolificacy of Iberian sows is more influenced by embryo losses in the first third of pregnancy than by ovulation rate.

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