Use of ultrasonography to detect puberty in gilts

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Introduction

In a group of gilts, the spontaneous occurrence of the first oestrus is generally spread over several weeks; within the Large White breed, a range around 15 weeks has been recorded between the earliest - and the oldest age (Desprès et al., 1992). Environmental and management conditions influence the timing of puberty (Evans, O'Doherty, 2001). The presence of the boar accelerates the occurrence of puberty (Brooks & Cole, 1970; Patterson et al., 2002) and when associated with transport stress, allows synchronisation of an increased proportion of first oestrus within a few days. However, the results of such procedures are highly variable (Martinat-Botté et al., 1985; Signoret et al., 1990).

So another method has been searched than the detection of oestrus by a boar to estimate the onset of puberty in gilts. Echography is selected because this method is able to visualise uterine evolution during early pregnancy and post-partum (Martinat-Botté et al., 2000). First, the changes in the uterus are monitored by ultrasonography prior to and around the time of puberty. Second, the ultrasonographic images are related to the physiological status: infantile, impubertal, prepubertal or pubertal (Martinat-Botté et al., 2003). Third, a diagnosis of puberty based on the difference of uterine images has been evaluated in practical conditions (Martinat-Botté et al., 2004).

1- Main mechanisms involved in attainment of puberty in gilts

During sexual development four periods have been described for the Large White females (Camous et al., 1985): 1) A perinatal phase characterized by the high secretion of LH and FSH. 2) An infancy period during the 2nd month of age. Secretion of LH is low while that of FSH remains high. 3) A phase of initiation of sexual development during the third and fourth months. Under the stimulation due to the increase of FSH followed by that of LH secretion, antral follicles appear in the ovaries and estrogen production begins to increase. 4) The last one or waiting period from the fifth month of age which is near puberty. This period has been characterised by a decrease in LH and FSH secretions and an increase in ovarian secretions which acts on uterine development and the central nervous system (Evans, O'Doherty, 2001; Elsaesser et al., 1998; Elsaesser et al., 1991). The growth of the uterus has been observed during this period (Dyck, 1988; Erices & Schnurrbusch, 1979; Prunier et al., 1987).

Variability in age at puberty and differences in hormone levels at a given age suggest that the duration of the different phases of this pattern varies between gilts.

2 - Basic elements of anatomy and topography of genital tract in gilts around puberty

a) Morphology and weight of uterus

Several physiological status can be identified (Martinat-Botté et al., 2000) at the genital tract observations of gilts 6 to 7 months of age: 1) infantile are those gilts whose uterus is still small (weight: <50g) and whose ovaries present smaller follicles (< 0.5 cm) and no other structures, 2) impubertal are those gilts whose uterus begin to grow (weight:

around 100g) and vascularise and whose ovaries present no other structures than follicles (between 0.1 and 0.6 cm), 3) prepubertal gilts which have uteri which are developed (weight: around 250g) and have a few follicles between 0.6 and 0.8 cm on the ovaries, 4) pubertal gilts which have a developed uterus (weight: >300g) and ovaries with corpora lutea and/or corpora albicantia and follicles.

The mean uterine weight varies significantly according to physiological status. For the pubertal gilts, the uterine weight is around 8 times heavier than that of the infantile gilts (Table 1).

Table 1: Age (days) and uterine weight (g) and uterine area (cm^2) of gilts at slaughter according to physiological status

Physiological status	Experiment 1				Experiment 2		
	Number of gilts	Age at slaughter (days)	Uterine weight (g)	Mean uterine area (cm^2)*	Number of gilts	Age at slaughter (days)	Uterine weight (g)
Infantile	2	181 - 182	46 - 50	3.5±2.3[h]	0	-	-
Impubertal	4	182±13[a]	120±11[b]	13±5.6[i]	55	201±7 [a]	118±59[e]
Prepubertal	1	184	291	30.2	9	201±7 [a]	258±75[f]
Pubertal	10	185±14[a]	359±18[c]	40.5±7.2[j]	9	210±8[d]	534±95[g]

* the day before slaughter; means (X ± SD) of the same row with different superscipt are different ($p<0.01$).

(From Martinat-Botté et al., 2003).

b) Topography of uterus

The topography of the genital tract also varies with the age (Martinat-Botté et al., 2000). Around 5 months of age, the uterus is located at the top of the bladder, near to the pelvic symphyse between the 3rd and the 1st sacral vertebra. It is thus far from the abdominal wall in a difficult zone to visualise it by echography. With the development of the uterus, the uterine horns are in front of the bladder and sometimes even under this one but remains located at the level of the sacral vertebrae. In this case, the uterus is easier to visualise.

c) Consequences on the ultrasound examinations

The examination procedure described for adult sows (Martinat-Botté et al., 2000) is

modified for 6 month-old gilts. The exploration is carried out externally by applying the linear probe (5MHz) only in the inguinal fold. The transmitting zone of the transducer is coated with contact gel. For the impubertal gilt, the probe is placed as high as possible in the inguinal zone and firmly pressed on the animal's skin to visualise the uterus. For the pubertal gilt, the transducer is placed in the inguinal fold and the uterus is rapidly visualised (Martinat-Botté et al., 2003).

The immobilization of the gilts into a crate adapted to the size of the gilts is necessary to obtain a better visualisation of the uterus. To prevent the gilts from moving during the examination, some feed is distributed.

3- Determination by echography of uterine changes around puberty

The experiment has been carried out to determine if uterine changes around puberty can be visualised by ultrasonography. The gilts are examined by echography 3 times a week from 130 to 185 days of age. For each gilt, 23 to 25 examinations are done during the experimental period. The detection of oestrus is performed once per day from 130 days of age with a boar. The ultrasonic appearance of the uterus is described, quantified and compared with the reproductive status observed at slaughter (around 180 days of age; Martinat-Botté et al., 2003).

a) Changes of "uterine area" around puberty

The images are recorded and the area occupied by the uterus in the image or "uterine area" is computed. Figure 1 shows the changes of "uterine area" during the echographic follow-up (54 days on average) for pubertal gilts (with or without oestrus) and for those which are infantile, impubertal and prepubertal at slaughter.

For infantile and impubertal gilts, the "uterine area" data do not increase during the follow-up. The uterine growth seems slow. At slaughter, the mean uterine weight is the lowest in immature gilts compared to pubertal ones (Table 1, Experiment 1).

For pubertal females whose oestrus is detected, the "uterine area" increased rapidly around 180 days old which is near puberty. Three gilts not seen in oestrus are pubertal at slaughter. For these females, an increase in "uterine area" is observed around 180 days of age. The same trend is noted for the only one prepubertal gilt at slaughter. This evolution is as a result of a marked increase in uterine horn weight and length observed near the onset of puberty.

Kauffold et al. (2004) have characterised the uterus by transcutaneous ultrasonography in gilts around puberty. For each gilt, the uterine horns are imaged in a cross-section and their diameter are measured. The mean sectional areas are calculated from the measured diameters. The mean sectional area is the lowest for impubertal animals compared to pubertal gilts with one or more sexual cycle. With a different parameter, "uterine area", we obtain similar results which are in agreement with those of Kauffold et al. (2004).

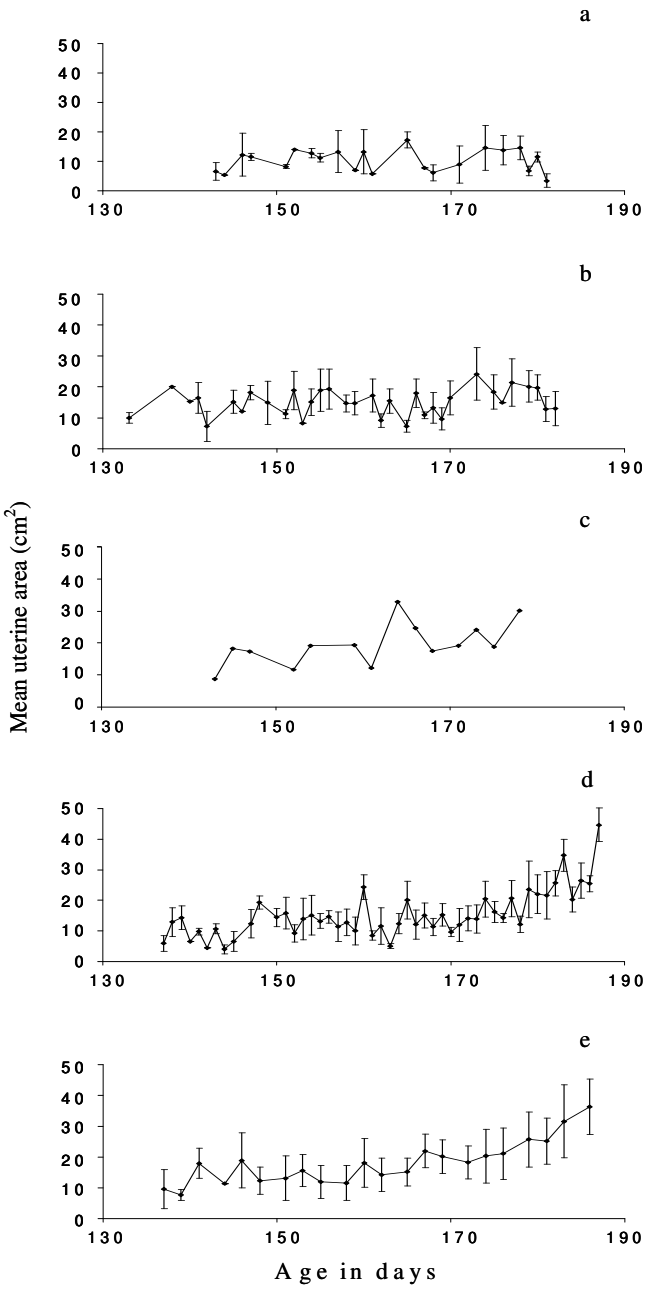

Figure 1: Evolution of "uterine area" in hyperprolific Large White gilts between 130 to 190 days old (X ± SD – except for c).
a) Infantile gilts at slaughter (n=2).
b) Impubertal gilts at slaughter (n=4).
c) Prepubertal gilt at slaughter (n=1).
d) Pubertal gilts seen in oestrus before slaughter (n=7).
e) Pubertal gilts not seen in oestrus before slaughter (n=3).

From Martinat-Botté et al., 2003.

b) Uterine changes in ultrasound images around puberty (Figure 2)

For infantile and impubertal gilts at slaughter, uterine images remained more and less dark and always homogeneous during the whole period (Photos 1,2). The uterus takes up much less place than the gut loops and bladder (Photos 1,2). The uterus is often restrained between the bladder and the intestinal mass (Photo 1). Sometimes, the uterus is

behind the bladder.

For pubertal gilts at slaughter, until 165 to 170 days old, the uterine images remained more and less dark and homogeneous. The same difficulties described above for immature gilts are encountered during this period. The uterine images change a few days before the onset of puberty. Well defined sections of the uterine horns are observed on the screen (Photos 3,4). Uterine sections are visualised under or near the bladder. This type of echographic images occurs nine to one day before puberty. In three gilts which have ovulated without showing oestrus, changes of the uterine images are also observed. For the only one prepubertal gilt at slaughter, the uterine sections are noted four days before. The ovaries with follicles are seen the day before slaughter.

The pattern of the development of "uterine area" is progressive and several quantitative measurements are required to be evidenced. The occurrence of well defined images of the uterus is sharp and always occurred a few days before puberty and remained after puberty. The difference of uterine images is also noted in gilts close to puberty - prepubertal females. Thus these changes are retained for an evaluation of a puberty diagnosis.

4- A practical use of a diagnosis of puberty determined by echography

From batch to batch, the onset of puberty is variable and is generally spread over several weeks (Desprès et al., 1992; Evans, O'Doherty, 2001; Dyck, 1988; Eliasson et al., 1991). For the breeders, such variability in the age of puberty is a problem for the introduction of the gilts in batches of farrowings. A diagnosis of puberty would facilitate entering into a breeding herd. A study has been carried out to evaluate over two years (March 2001 to January 2003) this new tool in a herd organized in batches every 3 weeks (Martinat-Botté et al., 2004). Gilts (n=687) of 197 ± 11 days (X ± SD) of age are examined at random by echography for the first time. For the gilts not observed in oestrus, the follow-up is continued at around 7-day intervals and 2 or 3 examinations more have been done. For the last echography (at 210 days in mean), one day after, the gilts are slaughtered and their genital tracts are observed. The physiological status is noted as described above and compared to the echography diagnosis.

a) Calculation mode of diagnosis accuracy

The echographic images of the uterus are codified as follows: females are noted + when well defined slices of the uterus are visualised and females are recorded - when the uterine images are dark and homogenous.

The results of the ultrasound examinations are compared to the physiological status of the gilts. There are four cases. 1) True positive (TP: ++) : the examination result is **+** and corresponds to gilts seen in oestrus or at slaughter to prepubertal and pubertal gilts and noted **+**. 2) False positive (FP: +-) : the examination result is **+** and corresponds at slaughter to infantile and impubertal gilts and noted **-**. In this case, we have considered that there is a false positive diagnosis. 3) True negative (TN: **--**) : the examination result is **-** and corresponds at slaughter to infantile and impubertal gilts and coded **-**. 4) False negative (FN: -+) : the examination result is negative and corresponds to gilts seen in oestrus or at slaughter to prepubertal and pubertal gilts and noted **+**. In this case, we have considered that there is a false negative diagnosis.

The sensitivity of the diagnosis is defined as the number of true positives (TP) as a proportion of all results from truly prepubertal and pubertal gilts (TP + FN) and given as percentage. The specificity of the test is defined as the number of correct negatives (TN) as

a proportion of the total test results for truly infantile and impubertal gilts (TN + FP) and expressed in percentage. The accuracy of diagnosis is calculated as the ratio between correct diagnoses and the number of diagnoses carried out, given in percentage.

b) Accuracy of the results of puberty diagnosis

At 210 days of age, 18% of gilts (n=687) are impubertal and 3% of them are prepubertal at slaughter. Among the pubertal females (n=536), 7% have ovulated without oestrus (slaughtered gilts). This percentage confirms the results from investigations carried out under field conditions which demonstrate high progesterone levels in pubertal gilts without oestrus behaviour. For these studies, from 5 to 20 percent of the gilts do not show a standing reflex at the first oestrus. All these gilts ovulate, as judged by the progesterone levels (Signoret et al., 1990; Christenson, 1981; Eliasson, 1989).

Among the 909 echographic examinations done, 20 of them are uninterpretable (2.2%). Generally the uterus is not visualised. These data are withdrawn of the analysis.

The sensitivity of the diagnosis is high: 99.2% (Table 2). This technique allows the detection of the most pubertal females which is the population that the pig breeders must detect with a reliable accuracy. Moreover, the ultrasound scanning permits to detect all the gilts (n=38) which ovulate without oestrus being detected. The implementation of ultrasound scanning is easier than the progesterone assay.

For the immature females, the specificity of the diagnosis is 92.1% (Table 2). The errors (less than 8%) are generally due to the appearance on the screen of "small" and "not well defined" sections of the uterine horns.

The overall diagnosis accuracy was 97% (Table 2).

Table 2: Diagnosis accuracy of puberty in Large White hyperprolific gilts in one herd (From Martinat-Botté et al., 2004).

Number of examinations	Diagnosis of puberty	(%)
622	Sensitivity	99.2
267	Specificity	92.1
889	Accuracy	97.0

Conclusion

The occurrence of puberty is a sudden event that is predictable by echography few days before the onset of oestrus. The difference of uterine images between pubertal and impubertal gilts can be used as an accurate diagnosis of puberty : 97% of accuracy. For pubertal gilts with or without oestrus detection the errors are less than 1%.

This diagnosis will facilitate the entry of gilts into sow groups when sows are bred after weaning. Hence, it opens up a new possibility.

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