A mixture model to assess the effect of a hormonal stimulation on left censored follicle growth curve in prepubertal heifers

Philippe Lambert

Université Catholique de Louvain, Institut de Statistique, Voie du Roman Pays 20,

B-1348 Louvain-la-Neuve, Belgium.

Summary. The goal of this study is to assess the effect of FSH (follicle stimulating hormone) on the growth of follicles in prepubertal heifers. Adams, Evans and Rawlings (1994) have shown that follicles develop in waves like in postpubertal heifers: after a few days, a dominant follicle arises on one of the two ovaries while the other subordinate follicles regress. The dominant follicle remains detectable when the next wave occurs. The number and the diameters of these follicles have been measured during 14 days.

The same experiment was repeated after a 3-day FSH stimulation. Several large follicles now arise simultaneously on both ovaries together with small follicles which cannot be detected anymore when the next wave occurs.

Unfortunately, follicles were not individually tracked. Therefore a three-component mixture model was used to describe the potential simultaneous observation of dominant (large), decaying dominant (large) and subordinate (small) follicles before (after) the FSH stimulation. Moreover, for technical reasons, only follicles larger than 2mm are observable. Measurements were thus left-censored.

The fitted models are used to quantify the effects of the stimulation and to determine the ideal time point at which follicles should be punctured under ultrasonography to harvest ovocytes that will be fertilised and cultured in vitro.

Key words: Mixture model; Left censoring; Growth curve.

---

email: lambert@stat.ucl.ac.be

1. Introduction

The purpose of the study is to quantify the effect of an hormonal stimulation on the number and the diameter of growing follicles arising on the right and left ovaries in prepubertal heifers.

Fourteen heifers have been observed daily over a period of 23 days. Follicles have been removed from both ovaries on day 0. The number and the diameter of detectable follicles on each ovary of the fourteen animals were thereafter recorded daily at 2 p.m. from day 1 to day 14 using transvaginal ultrasonography.

Follicles were again removed from both ovaries just after the collection of observations on day 14. The same records as above were obtained daily at 2 p.m. from day 15 to day 28. The follicle stimulating hormone (FSH) was injected at 8 a.m. and 8 p.m. on days 16, 17 and 18.

For technical reasons, veterinaries can only insure that they will be able to detect follicles with diameters of at least 2mm. Therefore, we shall ignore records corresponding to smaller entities as the technician might have missed other follicles of similar sizes.

The resulting data are plotted on Figures 1, 2, 3 and 4 for heifer 1641, 6039, 8140 and 9991 respectively.

[Figure 1 about here.]

[Figure 2 about here.]

We clearly see that the hormonal stimulation (on days 16-18) seems to increase both the diameters and numbers of subsequent follicles larger than 2mm on both ovaries.

[Figure 3 about here.]

[Figure 4 about here.]

Adams et al. (1994) have shown that transrectal ultrasonography was suitable for imaging the ovaries of prepubertal heifers. Their observations support the hypothesis that follicular development occurs in waves like in mature cattle. Waves occured ± 1 day after a 3-day FSH surge reached its maximum.

A wave of follicular growth implies the simultaneous development of a dominant follicle (on one of the ovaries) and several subordinates. This dominant follicle suppresses the growth of its subordinates. It remains anovulatory in prepubertal heifer.

The dominant follicle was distinguishable from its subordinates ± 2.5 days after the beginning of a wave. A period of ± 8 days was observed between two successive dominant follicles (from successive waves).

The same authors also noticed that the diameter of the largest follicle is inversely related to the number of follicles detected. They conclude that follicle development is qualitatively similar in pre- and postpubertal heifers.

A major problem with our dataset is that for practical reasons, the veterinary decided not to track individual follicle growth. Except with the dominant follicle when it is closed to its maximum diameter, it is nearly impossible to identify the growth curve for a specific follicle.

Therefore we shall heavily rely on (prior) information available in the literature on the development of prepubertal heifer follicles, our main source being Adams et al. (1994).

The plan of the paper is as follows. In Section 2, we propose an equation to describe the evolution of the mean diameter of a follicle. The components of a mixture and the probability to belong to any of them is described. A distinction is made between the before and the after FSH stimulation periods. In Section 3, the likelihood used to make inference on the effect of the stimulation on the follicle diameter is presented together with the underlying modelling strategy. The selected model is discussed and the first conclusions are drawn. In Section 4, we study the effect of the stimulation on the number of follicles detected during a wave using a Poisson-normal model (Hinde, 1982). We conclude the paper by a discussion in Section 5.

2. Basic growth curves

2.1 *Follicle growth before stimulation*

Based on Figure 2 in Adams et al. (1994), we propose to describe the growth curve of a follicle belonging to a given wave by

$$\mu(t|\mu^{(\max)}, \tau^{(\max)}) = \max\left\{0, \mu^{(\max)} \sin\left(\frac{\pi t}{2\ \tau^{(\max)}}\right)\right\} \qquad \text{with } t \in (0, 2\ \tau^{(\max)}) \tag{1}$$

where t, $\mu^{(\max)}$ and $\tau^{(\max)}$ respectively measure the time elapsed since the wave beginning, the maximum follicle size and the time required to observe that maximum.

When the ovaries are not stimulated, a dominant follicle usually arises on one of the two ovaries. It remains detectable when the next follicular wave occurs. Therefore, we must make a distinction between dominant and subordinate growth curves while keeping their shape similar. Thus different values for $\mu^{(\max)}$ and $\tau^{(\max)}$ will be allowed with an upper script "(d)" and "(s)" for dominant and subordinate follicles respectively. If n_1 is the number of emitted follicles in the 1st wave on an ovary and d_1 ($= 0$ or 1) the number of dominant follicles among n_1, the proportion of dominant and subordinate follicles is $p^{(d)}_{1,\neg\mathrm{Tx}} = d_1/n_1$; $p^{(s)}_{1,\neg\mathrm{Tx}} = 1 - p^{(d)}_{1,\neg\mathrm{Tx}}$ where "¬Tx" indicates that no treatment (stimulation) was applied.

But we only observe follicles larger than 2mm. Therefore, n_i will just be the number of emitted follicles which become detectable in the ith wave. The follicles that we observe in the first wave before treatment were necessarily emitted between day 0 and day 1. We shall make the assumption that the emission occured at time $t = 0.5$. The value of n_1 will be set equal to the largest number of follicles detected simultaneously during the first wave. This should happen approximately at $(T_2 - T_1)/2$, i.e. half way between the first and the second wave emission times. As Adams et al. (1994) observed ± 8 days between two successive dominant follicles, we expect to find n_1 approximately 4 days after the wave occurrence, i.e. around $t = 4.5$ for wave 1.

Let us now consider the second wave. The dominant follicle from wave 1 is still detectable on the ovary if it was observed ($d_1 = 1$). Therefore we have a possible mixture of a dominant (if $d_2 = 1$), a decaying dominant (if $d_1 = 1$) and subordinates follicles. The proportion of these types of follicles during the second wave is thus (respectively)

$$p^{(d)}_{2,\neg\mathrm{Tx}} = \frac{d_2}{n_2} \; ; \; p^{(d-)}_{2,\neg\mathrm{Tx}} = \frac{d_1}{n_2} \; ; \; p^{(s)}_{2,\neg\mathrm{Tx}} = \frac{n_2 - d_2 - d_1}{n_2}$$

2.2 *Follicle growth after stimulation*

The effect of a FSH stimulation is mainly to increase the number of developing and detectable follicles. We do not observe a dominant follicle anymore but rather several *large* follicles on both ovaries which are still apparent when the next wave occurs. The proportion of large and *small* (in opposition to large) follicles emitted per wave under stimulation will be denoted by $\pi^{(d)}$ and $\pi^{(s)} = 1 - \pi^{(d)}$. We shall assume that $\pi^{(d)}$ takes the same (unknown) value for all heifers. Therefore, the proportions $p_1^{(d)}$ and $p_1^{(s)}$ of large and small follicles during the first wave will just be $\pi^{(d)}$ and $\pi^{(s)}$.

During the second wave, the large follicles from the 1st wave are still detectable (by definition). Therefore we have a mixture of large, decaying large and small follicles. The proportion of these types of follicles are thus (respectively)

$$p^{(d)}_{2,\mathrm{Tx}} = \frac{(n_2 - n_1 p_1^{(d)})\pi^{(d)}}{n_2} \; ; \; p^{(d-)}_{2,\mathrm{Tx}} = \frac{n_1 p_1^{(d)}}{n_2} \; ; \; p^{(s)}_{2,\mathrm{Tx}} = \frac{(n_2 - n_1 p_1^{(d)})\pi^{(s)}}{n_2}$$

where "Tx" indicates that a treatment (stimulation) was applied.

The same growth curve as before stimulation (cf. Equation (1)) will be considered here. The stimulation will be assumed to have only an effect on $\mu^{(\max)}$, $\tau^{(\max)}$ and wave frequencies.

3. Statistical modelling of the follicle diameter

After the qualitative description of follicular wave patterns, let us study the effect of the FSH stimulation on the follicle diameters. We shall work conditionally on the maximum number of follicles (larger than 2mm) observed simultaneously during each wave on each of the ovary of the fourteen heifers. Each of these numbers will be interpreted as the number of different follicles (which reach a diameter of at least 2mm) detected on a given ovary of a given heifer during a given follicular wave. It is obviously not totally correct, but this should be a reasonable approximation as confirmed by a discussion with the involved veterinary.

3.1 *Likelihood expression and description*

The likelihood function that we shall use to make inference on the follicle diameters is

$$
\begin{aligned}
&\prod_{\mathrm{Tr}} \prod_{\text{heifer } i} \prod_{\text{ovaries } v} \prod_{\text{waves } w} \prod_{\text{local time } t} \\
&\quad \Big\{ p_{w,\mathrm{Tr}}^{(s)}\, F\left(2 \,\middle|\, \mu(t|\mu_{\mathrm{Tr}}^{(s)}, \tau_{\mathrm{Tr}}^{(s)}), \phi_{\mathrm{Tr}}^{(s)}\right) + p_{w,\mathrm{Tr}}^{(d)}\, F\left(2 \,\middle|\, \mu(t|\mu_{\mathrm{Tr}}^{(d)}, \tau_{\mathrm{Tr}}^{(d)}), \phi_{\mathrm{Tr}}^{(d)}\right) \\
&\qquad + I(w>1)\, p_{w,\mathrm{Tr}}^{(d-)}\, F\left(2 \,\middle|\, \mu(t + T_{\mathrm{Tr}}|\mu_{\mathrm{Tr}}^{(d)}, \tau_{\mathrm{Tr}}^{(d)}), \phi_{\mathrm{Tr}}^{(d)}\right) \Big\}^{N_{ivw,\mathrm{Tr}} - n_{ivwt,\mathrm{Tr}}} \\
&\quad \prod_{j=1}^{n_{ivwt,\mathrm{Tr}}} \Big\{ p_{w,\mathrm{Tr}}^{(s)}\, f\left(d_{ivwtj,\mathrm{Tr}} \,\middle|\, \mu(t|\mu_{\mathrm{Tr}}^{(s)}, \tau_{\mathrm{Tr}}^{(s)}), \phi_{\mathrm{Tr}}^{(s)}\right) + p_{w,\mathrm{Tr}}^{(d)}\, f\left(d_{ivwtj,\mathrm{Tr}} \,\middle|\, \mu(t|\mu_{\mathrm{Tr}}^{(d)}, \tau_{\mathrm{Tr}}^{(d)}), \phi_{\mathrm{Tr}}^{(d)}\right) \\
&\qquad\qquad + I(w>1)\, p_{w,\mathrm{Tr}}^{(d-)}\, f\left(d_{ivwtj,\mathrm{Tr}} \,\middle|\, \mu(t + T_{\mathrm{Tr}}|\mu_{\mathrm{Tr}}^{(d)}, \tau_{\mathrm{Tr}}^{(d)}), \phi_{\mathrm{Tr}}^{(d)}\right) \Big\} \qquad (2)
\end{aligned}
$$

where

- Tr indicates whether no hormonal stimulation (Tr = "$\neg$Tx") or a stimulation (Tr = "Tx") was applied ; i ($i = 1, \ldots, 14$) indexes heifers; $v \in \{R, L\}$ indicates if we observe the right or the left ovary; $w \in \{1, 2, \ldots\}$ indexes the waves; t gives the time elapsed since the beginning of the wave.

- f and F are the density and distribution functions of a follicle diameter. They are presented here with a location and a dispersion parameters. The location parameter will be named the mean to simplify the discussion below, although it is not a requirement. One might also wish to consider three or four parameter distributions.

- $d_{ivwtj,\mathrm{Tr}}$ is the diameter of the jth follicle on the ovary v of heifer i observed after t days in wave w under treatment Tr.
- $N_{ivw,\mathrm{Tr}}$ is the maximum number of follicles observed on ovary v of heifer i in wave w under treatment Tr.
- $n_{ivwt,\mathrm{Tr}}$ is the number of follicles observed on ovary v of heifer i under treatment Tr after t days in wave w.
- $\mu_{\mathrm{Tr}}^{(s)}$ and $\mu_{\mathrm{Tr}}^{(d)}$ are (respectively) the maximum mean diameter of a subordinate and a dominant follicle under treatment Tr.
- $\tau_{\mathrm{Tr}}^{(s)}$ and $\tau_{\mathrm{Tr}}^{(d)}$ are (respectively) the times required for the mean diameter of a subordinate and dominant follicle to reach their maximum value $\mu_{\mathrm{Tr}}^{(s)}$ and $\mu_{\mathrm{Tr}}^{(d)}$ (respectively) under treatment Tr. These times are thus assumed to be the same for all heifers.
- $\phi_{\mathrm{Tr}}^{(s)}$ and $\phi_{\mathrm{Tr}}^{(d)}$ are (respectively) the values of the dispersion parameter for the subordinate and dominant follicle diameter distribution under treatment Tr
- T_{Tr} is the number of days separating two consecutive waves under treatment Tr. We make the assumption that these periods are the same for all heifers. This is of course a restriction, but it should not have too much consequence on the subsequent inferences as the possible heterogeneity of the T_{Tr}'s is usually small.

Thus, the contribution to the likelihood for the follicles observed after t days in wave w on ovary v of heifer i under treatment Tr is made of two pieces:

1. the probability for the diameters of the observed $n_{ivwt,\mathrm{Tr}}$ follicles to take their recorded values.

2. the probability for the other $N_{ivw,\mathrm{Tr}} - n_{ivwt,\mathrm{Tr}}$ follicles to be undetectable (i.e. smaller than 2mm) at time t.

Each of these pieces is a mixture of possibly three components:

1. the (weighted) probability for a subordinate (Tr $=$ "$\neg$Tx$''$) or small (Tr $=$ "Tx$''$) follicle diameter to be smaller than 2mm in the second piece or equal (after multiplication by the measurement precision, here $\Delta = 0.1$mm) to the recorded value $d_{ivwtj,\mathrm{Tr}}$.

2. the same expression for a dominant (Tr $=$ "$\neg$Tx$''$) or large (Tr $=$ "Tx$''$) follicle emitted during the considered wave.

3. the same expression for a dominant (Tr $=$ "$\neg$Tx$''$) or large (Tr $=$ "Tx$''$) follicle emitted during the previous wave if $w > 1$. The number of days elapsed since its emission is then $t + T_{w-1}$. Note that such a follicle cannot be observed during the first wave as all follicles were removed from the ovaries at day 0 and day 14.

The dispersion parameters $\phi^{(s)}_{\mathrm{Tr}}$ and $\phi^{(d)}_{\mathrm{Tr}}$ were allowed to take different values for subordinate (or small) and dominant (or large) follicles. It measures the possible variability of the follicle diameter around the mean value (cf. location parameter). The dispersion is assumed constant over time and similar for heifers under the same treatment.

The maximum value taken by the mean diameter, $\mu^{(s)}_{\mathrm{Tr}}$ and $\mu^{(d)}_{\mathrm{Tr}}$ for a subordinate (or small) and a dominant (or large) follicle respectively, is just allowed to vary with treatment. The possible heterogeneity between heifers follicle mean diameters was ignored. There are too many heifers to consider a fixed effect model and the complexity of the data (mixture, left censoring ...) does not allow to incorporate random effects easily. The same hypothesis was made for the times $\tau_{\mathrm{Tr}}^{(s)}$ and $\tau_{\mathrm{Tr}}^{(d)}$ at which these maxima are observed: these are just allowed to vary with treatment and follicle type. That assumption is similar to the homogeneity hypothesis made on the periods T_{Tr}.

3.2 *Modelling strategy*

The model underlying the likelihood in Equation (2) together with the hypotheses made in Section 3.1 is the most complex description of the process that we are ready to consider. We shall now fit that model to our data and see if further simplifying assumptions can be made.

The likelihood is conditional on a given wave structure or equivalently on $T_{\neg\mathrm{Tx}}$ and T_{Tx}. These quantities determine the number of observed waves on the periods $[0, 14]$ and $]14, 23]$ days together with the number $N_{ivw,\mathrm{Tr}}$ of different detectable follicles during a wave.

We have considered separately the grid of values $\{4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5\}$ days for $T_{\neg\mathrm{Tx}}$ and T_{Tx} under the assumption that the first waves without and under hormonal stimulation occured at days 0.5 and 14.5 respectively. The largest likelihoods were obtained under the hypothesis that $T_{\neg\mathrm{Tx}}$ for untreated and T_{Tx} for treated heifers are respectively equal to 7.5 and 6.5 days. Therefore, we conclude that the hormonal stimulation seems to shorten the wave frequency. This is in agreement with the a priori expectation of the veterinary. Indeed, it is reported in the literature that waves occur ± 1 day after a 3-day FSH surge reached its maximum. The last FSH injection was on day 18 at 8 p.m. Therefore, a wave should arise around day 20, i.e. earlier than after

- the reported (in the literature) 8 days separating two successive waves.
- the 7.5 days separating two successive waves before hormone stimulation.

Thus we shall now work conditionally on the following wave pattern:

	No stimulation (Tr = ¬Tx)		Stimulation (Tr = Tx)	
Wave w	1	2	1	2
Days	[0.5,8.0]	]8.0,14.0]	[14.5,21.0]	]21.0,23.0]

The values of the $N_{ivw,\mathrm{Tr}}$'s giving the numbers of different follicles detectable in the wave w of heifers 544, 547, 8732 and 8737 can be obtained from the lower graphs of Figures 1, 2, 3 and 4. These are summarized in Table 1.

[Table 1 about here.]

A normal distribution F was considered for the follicle diameters. It was set equal to zero for negative arguments as a diameter must always be positive. Other distributions like the Weibull and the gamma were also considered. No improvement was obtained in the global fit (as measured by the log-likelihood). Moreover, the conclusions related to treatment effect were not affected by the chosen distribution. Therefore, only the results associated to the truncated normal distribution will be reported here.

3.3 *Fitted models and interpretation*

The maximum likelihood estimates (MLEs) of the parameters in our reference model can be obtained by maximising the likelihood in Equation (2). These can be found in Table 2 together with the MLEs of the reduced model. We can see that the only possible simplifications consist in assuming

- the times $\tau^{(s)}_{\neg\mathrm{Tx}}$ and $\tau^{(s)}_{\mathrm{Tx}}$ required for respectively a subordinate and a small follicle to reach their maximum diameters

- the mean maximum diameters $\mu^{(d)}_{\neg\mathrm{Tx}}$ and $\mu^{(d)}_{\mathrm{Tx}}$ for respectively a dominant and a large follicle

to be the same. But note that the second simplification might be due to the small number of dominant follicles in the study.

[Table 2 about here.]

Based on that reduced model, we conclude the following:

- the mean maximum diameter $\mu_{\text{Tx}}^{(s)}$ of small follicles is significantly larger than the mean maximum diameter $\mu_{\neg\text{Tx}}^{(s)}$ of subordinate follicles. Combined with the previous conclusion related to dominant and large follicles, we see that the effect of the FSH stimulation is just detectable on the diameters of the smallest follicles.

- the dispersion $\phi_{\neg\text{Tx}}^{(s)}$ of the small follicle diameters is significantly smaller than the dispersion $\phi_{\text{Tx}}^{(s)}$ of the subordinate follicle diameters. The same is true when we compare the dispersion of large and dominant follicle diameters. Therefore, stimulating the ovaries might improve the regularity of the developing follicles as their diameters are more homogeneous than before the stimulation.

- The time $\tau_{\text{Tr}}^{(d)}$ (elapsed since the beginning of the wave) required for a large follicle to reach its maximum diameter is significantly smaller than the corresponding time $\tau_{\neg\text{Tr}}^{(d)}$ for a dominant follicle.

- the proportion $\pi^{(d)}$ of large follicles is estimated to be 0.73 (95% confidence interval $[0.65, 0.79]$). Therefore, a large majority of the detected follicles under stimulation are still larger than 2mm when the next wave occurs.

The evolution over time of the distribution of follicle diameters is illustrated on Figure 5 by a plot of their quartiles. These graphs summarise the above conclusions.

[Figure 5 about here.]

4. Statistical modelling of the numbers of detected follicles

In Section 3, we have quantified the effect of the FSH stimulation on follicle diameters conditionally on the numbers of different follicles detectable during a wave on each ovary of each heifer. These quantities were approximated by the largest number $N_{ivw,\mathrm{Tr}}$ of follicles observed simultaneously during the corresponding wave, ovary and heifer combination. Thus, these numbers were treated as if they were fixed given quantities.

Now, we propose to assess the effect of treatment on these numbers $N_{ivw,\mathrm{Tr}}$. A subset of these data can be found in Table 1. A Poisson model with a normal random effect (Hinde, 1982) was considered to account for the possible heterogeneity in the ovary behaviours:

$$\begin{aligned} N_{ivw,Tr} &\sim \mathrm{Pois}(\mu_{ivw,Tr}) \\ \log(\mu_{ivw,Tr}) &= \beta_0 + \delta_{\mathrm{Tr}} + \alpha_{iv} \quad ; \quad \delta_{\neg\mathrm{Tx}} = 0 \\ \alpha_{iv} &\sim N(0, \sigma^2) \end{aligned} \tag{3}$$

Parameter estimation was performed using Proc NLMIXED in SAS©, version 8. The corresponding parameter estimates can be found with their standard errors in Table 3.

[Table 3 about here.]

As can be concluded using the Akaike information criterion (AIC, Akaike, 1973), there is strong evidence that

- a random effect is required (heterogeneity between ovaries).
- the stimulation tends to increase the number of detectable follicles on an ovary: the mean number of detectable follicles is approximately increased by 43% (as $\exp(\hat{\delta}_{\mathrm{Tx}}) = 1.43$) [The corresponding 95% confidence interval is $(1.25, 1.64)$.] with a mean number of 6.62 ($= \exp(\hat{\beta}_0)$) detectable follicles before stimulation.

We can go further and distinguish the treatment effect during the first and the second wave after the stimulation. Indeed, as mentioned earlier, there is no extra FSH stimulation before the occurrence of wave 2. Therefore, we expect a very low level of that hormone in the blood at the beginning of the 2nd wave. The corresponding model is the same as in Equation (3), except that the equation for the mean is

$$\log(\mu_{ivw,\mathrm{Tx}}) = \beta_0 + \delta_{\mathrm{Tx},w} + \alpha_{iv}$$

for treated heifers. The corresponding parameter estimates can be found with their standard errors in Table 4.

Again, using the AIC, we conclude that

- a random effect is required.
- the mean number of follicles in the first wave after stimulation is significantly larger than before stimulation with a 64% (as $\exp(\hat{\delta}_{\mathrm{Tx},1}) = 1.64$) increase.
- the mean number of follicles in the second wave after stimulation is significantly
  - smaller than during the first wave after stimulation.
  - larger than before stimulation with approximately 23% (as $\exp(\hat{\delta}_{\mathrm{Tx},2}) = 1.23$ with 95% confidence interval $(1.04, 1.45)$) extra follicles. However, this does not mean that more follicles than before stimulation are produced during that second wave. Indeed, we know from Section 3.3 that about 73% ($= \hat{\pi}^{(d)}$) of the produced follicles during the first wave are large and therefore still detectable during the second wave. Thus, under the assumption that no treatment effect is detectable anymore on the number of emitted follicles during the second wave, we expect to observe during the second wave about

$$\exp(\hat{\beta}_0)[1 + \hat{\pi}^{(d)} \exp(\hat{\delta}_{\mathrm{Tx},1})] = 14.5$$

different follicles. This is about equal to the expected

$$\exp(\hat{\beta}_0)[1 + \exp(\hat{\delta}_{\mathrm{Tx},2})] = 14.7$$

number of follicles during the second wave. Thus, the apparent treatment effect on the number of detectable follicles during the second wave after stimulation is just attributable to the decaying large follicles from the first wave.

[Table 4 about here.]

5. Discussion

The major difficulty in the analysis of the presented dataset resides in the non identification of the observed follicles. Fortunately, a detailed qualitative description of the observed data generating process was available in the veterinary literature where an individual follow up of the follicles was undertaken. Based on this reliable prior information, we have set up a mixture model with potentially three components.

Stimulating the ovaries was obviously interesting as a majority of large follicles is now appearing on both ovaries instead of just an isolated dominant follicle.

We have analysed the follicles diameters associated to the second wave after treatment as if the second wave was totally comparable to the first one. This is of course a strong hypothesis. Indeed, as no stimulation was performed at the end of the first wave, the amount of FSH in the blood is certainly smaller a bit before and during the second wave than before and during the first wave. That was confirmed at the end of Section 4 when analysing the apparent treatment effect on the mean number of detectable follicles during this last wave. Therefore, we have probably underestimated the effect of the hormonal stimulation on the diameter of the detectable follicles. However, that underestimation should not be too large as during the approximately three days of observation of the 2nd wave after stimulation (i.e. after two days in this wave), there is practically no difference between subordinate (small) and dominant (large) follicles. Adams et al. (1994) report that the dominant follicle can be distinguished from its subordinates only ± 2.5 days after the beginning of a wave.

The presented models for the follicle diameters could obviously be improved by paying more attention to the potential heterogeneity between heifers or even between ovaries. However, the technical difficulties implied by such an extension are very important in the present case.

Let us conclude this discussion by giving some advise to the veterinary who just wish to know how many days after the beginning of the cycle he should collect follicles on the stimulated ovaries. A day during the first wave after stimulation should obviously be considered as the number of detectable follicles is the largest during that period. Moreover, about 73% of the detectable follicles are then large and thus more promising for further use in artificial insemination. As the estimated time $\hat{\tau}_{\mathrm{Tr}}^{(d)}$ (elapsed since the beginning of the wave) required for a large follicle to reach its maximum diameter is 5.81 (days) (with 95% confidence interval $[5.58, 6.04]$), it is probably advisable to sample the ovaries a bit before that number of days (cf. Figure 5), say after 5.5 days.

Acknowledgements

Research support from the contract "Projet d'Actions de Recherche Concertées" (PARC No. 98/03-217) of the Belgian Government is gratefully acknowledged.

Résumé

L'objectif de cette étude est d'évaluer l'effet de la FSH (follicle stimulating hormone) sur la croissance des follicules de génisses prépubères. Adams, Evans and Rawlings (1994) ont montré que les follicules apparaissent sous forme de vagues comme chez les génisses

postpubères: après quelques jours, un follicule dominant se distingue sur un des deux ovaires provoquant la dégénérescence des follicules subordonnés. Le nombre et les diamètres de ces follicules ont été mesurés pendant 14 jours.

La même expérience a été répétée après une stimulation hormonale de trois jours. Plusieurs grands follicules apparaissent simultanément sur les deux ovaires en même temps que des petits follicules. Contrairement aux grands follicules, ces derniers ne sont plus détectables lors de la vague suivante.

Malheureusement, les follicules n'ont pas été suivis individuellement. Nous avons donc utilisé un modèle de mélange à trois composantes pour décrire le développement potentiel et simultané de follicules dominant (grands), dominant (grands) de la vague précédente et subordonnés (petits). De plus, pour des raisons techniques, seuls les follicules d'un diamètre supérieur à 2mm peuvent être observés. Les données relatives au diamètre sont donc censurées à gauche.

Les modèles ajustés sont utilisés pour quantifier les effets de la stimulation sur le diamètre et le nombre de follicules observés, et pour déterminer le moment idéal où les follicules devraient être ponctionnés afin de récolter les ovocytes en vue d'une fécondation suivie d'une culture in vitro.

References

Adams, G., Evans, A. and Rawlings, N. (1994). Follicular waves and circulating gonadotrophins in 8-month-old prepubertal heifers. *Journal of Reproduction and Fertility* **100**, 27–33.

Akaike, H. (1973). Information theory and an extension of the maximum likelihood principle. In Petrov, B. and Csáki, F., editors, *Second International Symposium on Inference Theory, Budapest*, pages 267–281.

Hinde, J. (1982). Compound poisson regression models. In Gilchrist, editor, *GLIM82*, pages 109–121. Springer Verlag.

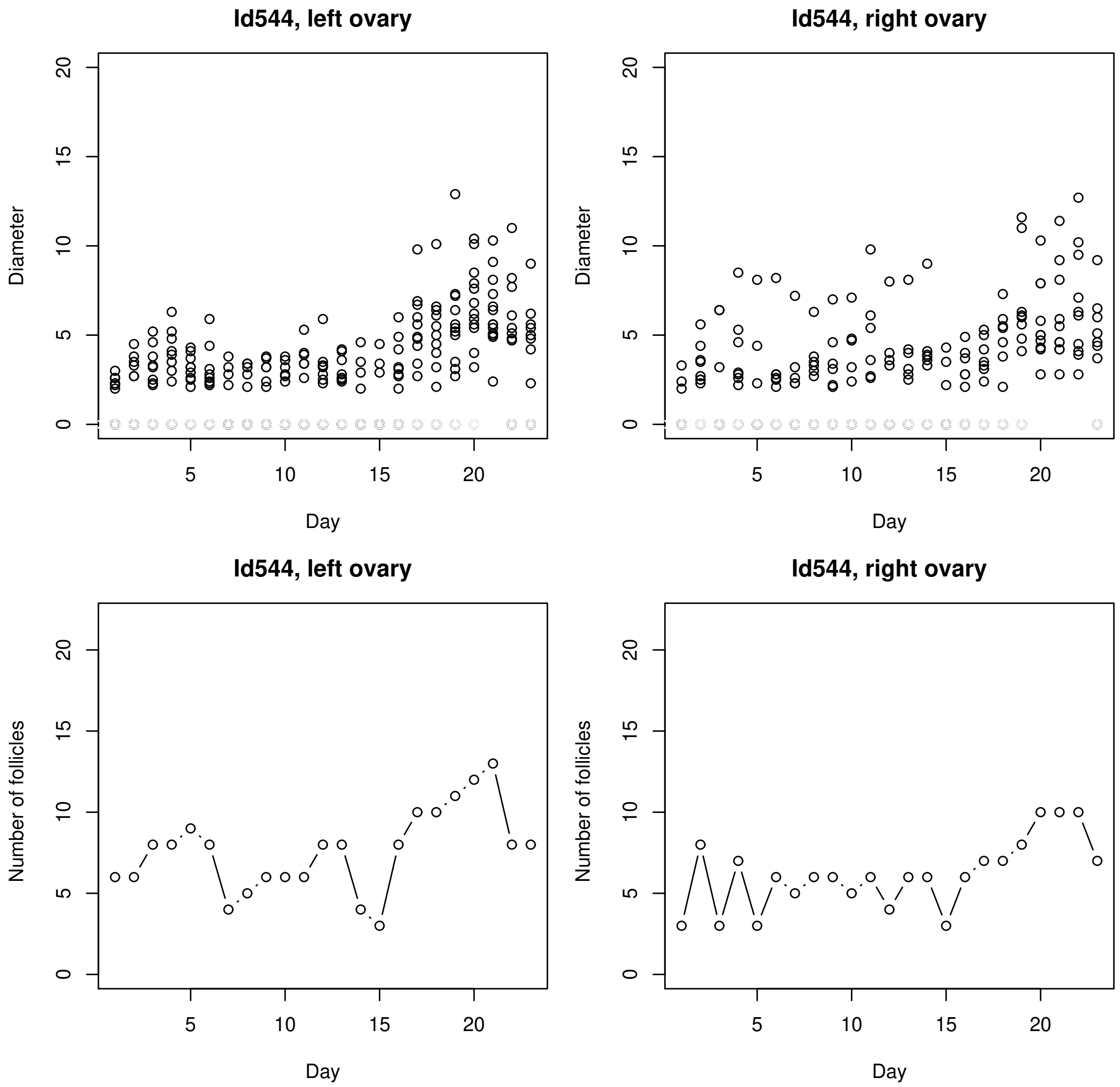

Figure 1. Diameter and number of follicles on left and right ovaries of heifer 544.

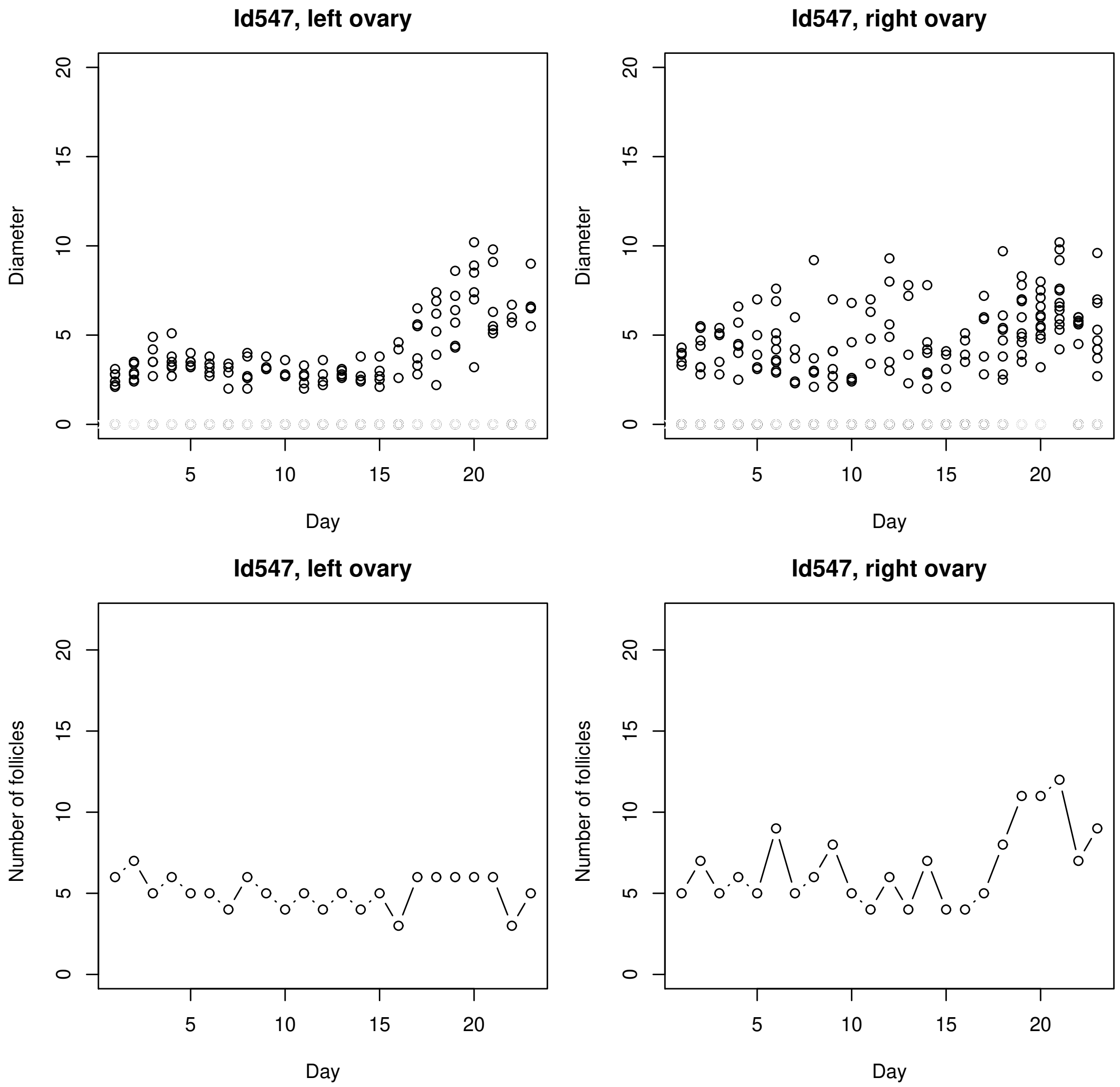

Figure 2. Diameter and number of follicles on left and right ovaries of heifer 547.

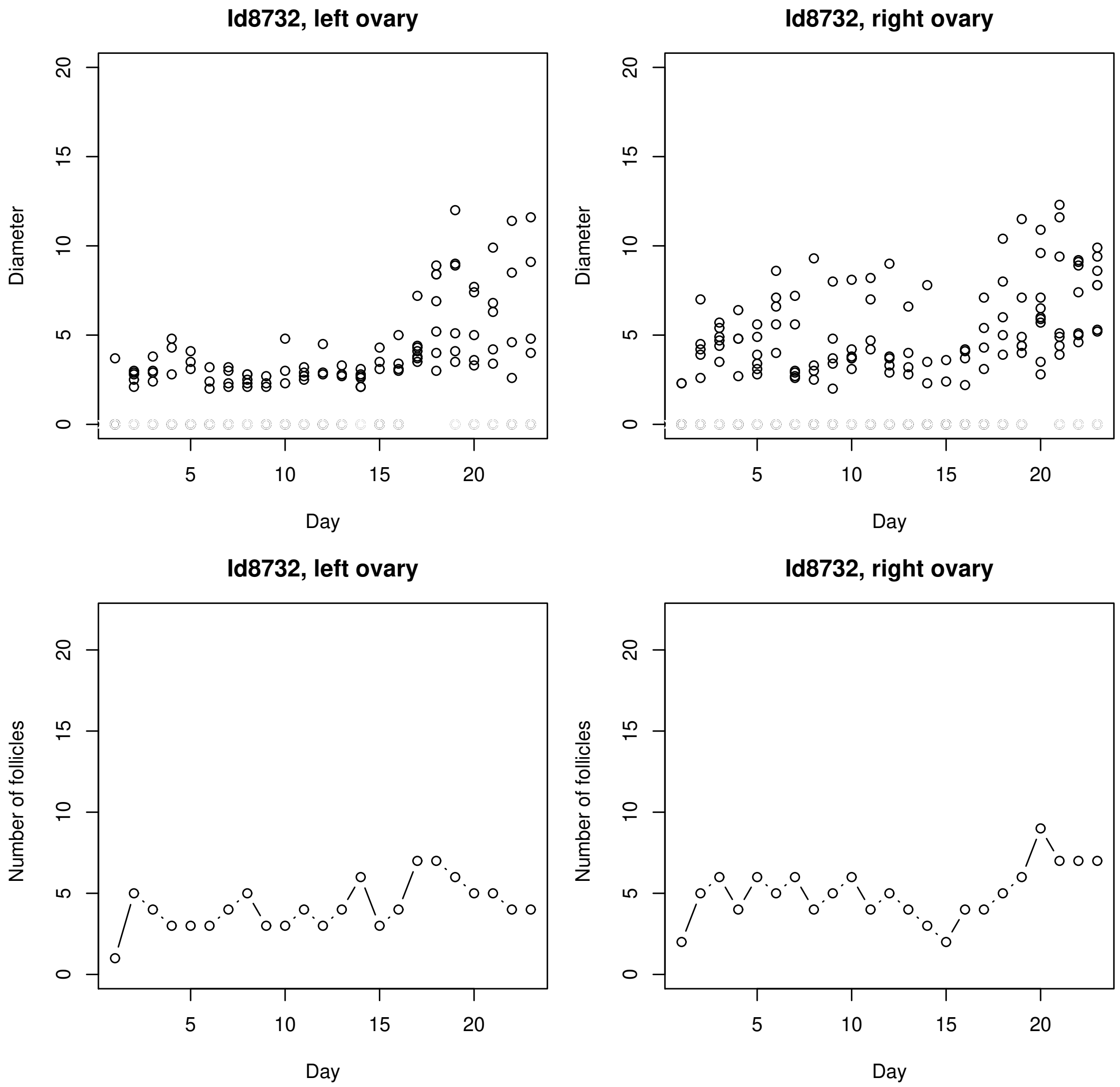

Figure 3. Diameter and number of follicles on left and right ovaries of heifer 8732.

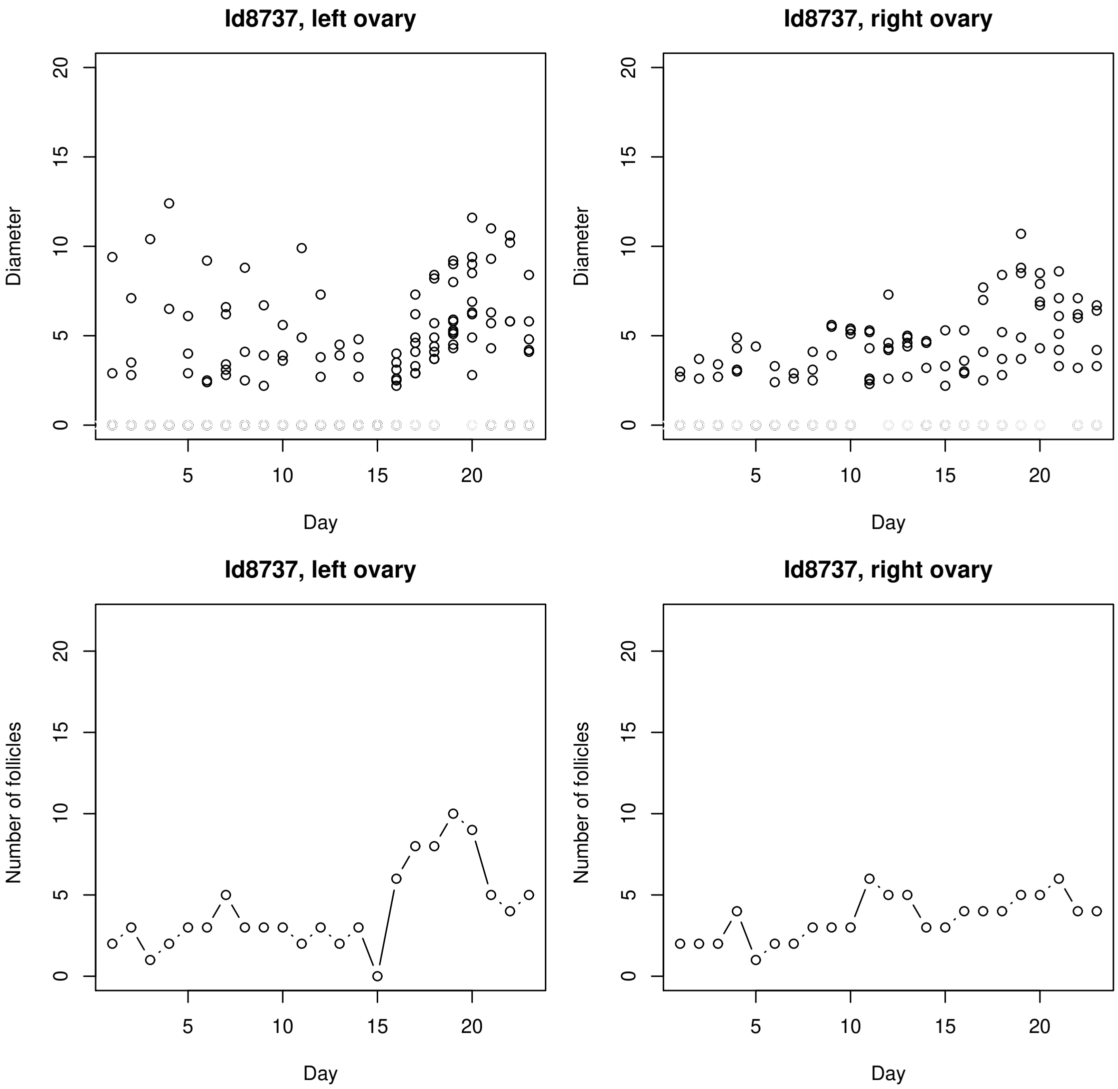

Figure 4. Diameter and number of follicles on left and right ovaries of heifer 8737.

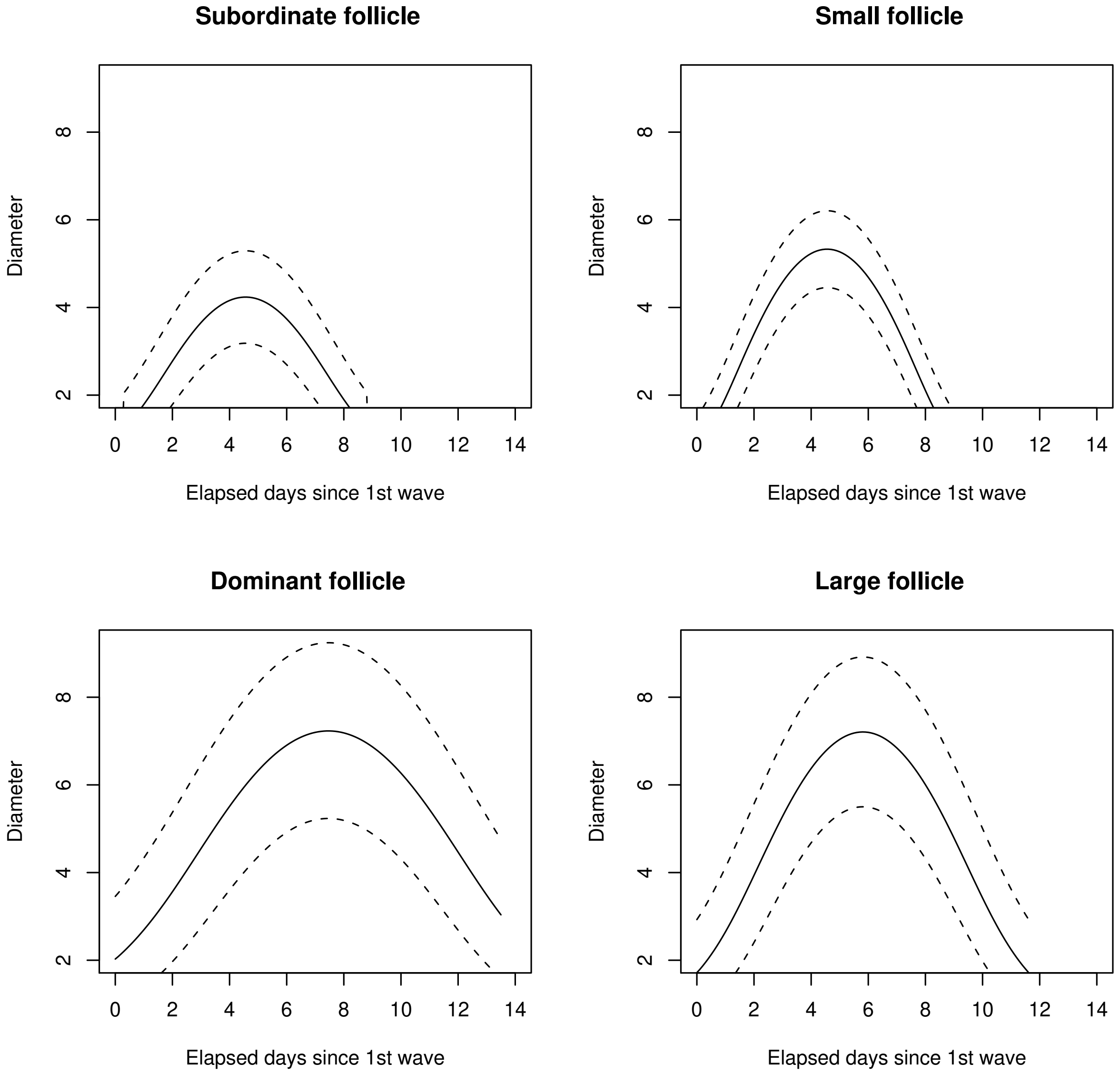

Figure 5. Quartiles of the fitted truncated normal distribution for the follicle diameter associated to a wave. The left and the right figures correspond, respectively, to untreated and treated heifers.

	No stimulation (Tr = ¬Tx)				Stimulation (Tr = Tx)			
	Left ovary		Right ovary		Left ovary		Right ovary	
	Wave 1	Wave 2	Wave 1	Wave 2	Wave 1	Wave 2	Wave 1	Wave 2
Heifer 544	9	8	8	6	13	8	10	10
Heifer 547	7	5	9	8	6	5	12	9
Heifer 8732	5	6	6	6	7	4	9	7
Heifer 8737	5	3	4	6	10	5	6	4

Table 1

Numbers of different follicles detectable during each of the waves observed on heifers 544, 547, 8732 and 8737.

	Full model		Reduced model	
Treatment	Tr = ¬Tx	Tr = Tx	Tr = ¬Tx	Tr = Tx
	Follicle type		Follicle type	
Parameter	Subordinate	Small	Subordinate	Small
$\tau_{\mathrm{Tr}}^{(s)}$	4.55 (0.089)	4.61 (0.201)	4.56 (0.081)	
$\mu_{\mathrm{Tr}}^{(s)}$	4.22 (0.080)	5.34 (0.305)	4.23 (0.080)	5.33 (0.277)
$\phi_{\mathrm{Tr}}^{(s)}$	1.56 (0.056)	1.30 (0.160)	1.57 (0.056)	1.30 (0.152)
	Follicle type		Follicle type	
Parameter	Dominant	Large	Dominant	Large
$\tau_{\mathrm{Tr}}^{(d)}$	7.33 (0.495)	5.82 (0.121)	7.46 (0.536)	5.81 (0.117)
$\mu_{\mathrm{Tr}}^{(d)}$	7.78 (0.487)	7.13 (0.186)	7.20 (0.175)	
$\phi_{\mathrm{Tr}}^{(d)}$	2.85 (0.262)	2.55 (0.074)	3.00 (0.255)	2.54 (0.074)
$\pi^{(d)}$	$\times$	0.74 $[0.66, 0.81]$	$\times$	0.73 $[0.65, 0.79]$
$-\log(\mathrm{Lik})$	7873.38		7874.96	
# Parameters	13		11	
AIC	15772.76		15771.92	

Table 2

MLEs (standard errors) and likelihood associated to the full and the reduced models.

	Poisson model		**Poisson-normal model**	
Parameter	No Tx effect	Tx effect	No Tx effect	Tx effect
β_0	2.116 (0.033)	1.920 (0.051)	2.086 (0.057)	1.890 (0.069)
δ_{Tx}	–	0.359 (0.067)	–	0.359 (0.067)
σ	–	–	0.244 (0.049)	0.244 (0.049)
# Par.	1	2	2	3
AIC	596.69	569.2	572.6	545.1

Table 3

MLEs (s.e.) associated to the Poisson and the Poisson-normal models for the number of detectable follicles per heifer/ovary/wave combination.

	Poisson model	**Poisson-normal model**
Parameter	Tx-wave effect	Tx-wave effect
β_0	1.920 (0.051)	1.890 (0.069)
$\delta_{\mathrm{Tx-Wave1}}$	0.493 (0.076)	0.494 (0.076)
$\delta_{\mathrm{Tx-Wave2}}$	0.203 (0.083)	0.203 (0.083)
σ	–	0.244 (0.049)
# Par.	3	4
AIC	559.8	535.6

Table 4

MLEs (s.e.) associated to the Poisson and the Poisson-normal models for the number of detectable follicles per heifer/ovary/wave combination when we consider different treatment effects for wave 1 and wave 2 after stimulation.