# Chapter 4
## *Molecular studies of baboon endometrial tissues*

# 4 Quantitative real time polymerase chain reaction (qRT-PCR) in baboon endometrial tissues

## 4.1 Introduction

In this chapter, we use the same rationale as in Section 3.1 to investigate whether NFκβ mRNA is present in baboon endometrial tissues. However, we were unable to look at the mRNA transcripts for PA28, IKKα and NFκβ in both the eutopic and ectopic endometrial tissues of baboons and conduct verification experiments. We therefore only examined whether the NFκβ mRNA transcript is absent within endometriotic tissues of the baboon animal model, mimicking the result found in humans in Chapter 3 that may indicate that a different pathway to NFκβ is possibly responsible for ectopic endometrial cell survival.

## 4.2 Materials and methods

### *4.2.1 Baboon tissues for qRT-PCR*

Lyophilised tissues for qRT-PCR were provided by Professor Asgi Fazleabas from the Department of Obstetrics and Gynaecology, The University of Illinois at Chicago, Chicago, USA and processed as described in Section 2.3.1. RNA quality and quantity was then measured according to the instructions in Section 2.3.4, using the Agilent Technologies $RNA_{6000}$ nano chip. Agilent recommends that an RIN of ≥ 7.0 is used for qRT-PCR analysis, however, samples with an RIN = 6.0, such as the one shown in Figure 4.1 were included. See representative gel in Figure 4.2 for RNA quality and quantity from baboons. cDNA was amplified as described in Section 2.3.5 by using primers designed according to Section 2.3.6 and listed in Table 2.3. An Invitrogen Platinum® SYBR® Green qPCR Super Mix-UDG kit and a Corbett Rotor-Gene™ 6000 real-time analyser as noted in Section 2.3.7 were utilised for qRT-PCR, where the number of amplicon products generated for NFκβ was measured. Standard curves were created and statistical analysis conducted for NFκβ normalised against the 18S

housekeeping gene as discussed in Section 2.3.8. See representative standard curve Figure 4.3.

## 4.3 Results

| **Table 4.1. Baboon mRNA expression of NFκβ transcript within the ectopic endometrium normalized for 18S** | |
|---|---|
| **Gene** | **Ectopic (n=3)** |
| NFκβ/18S | 0 |

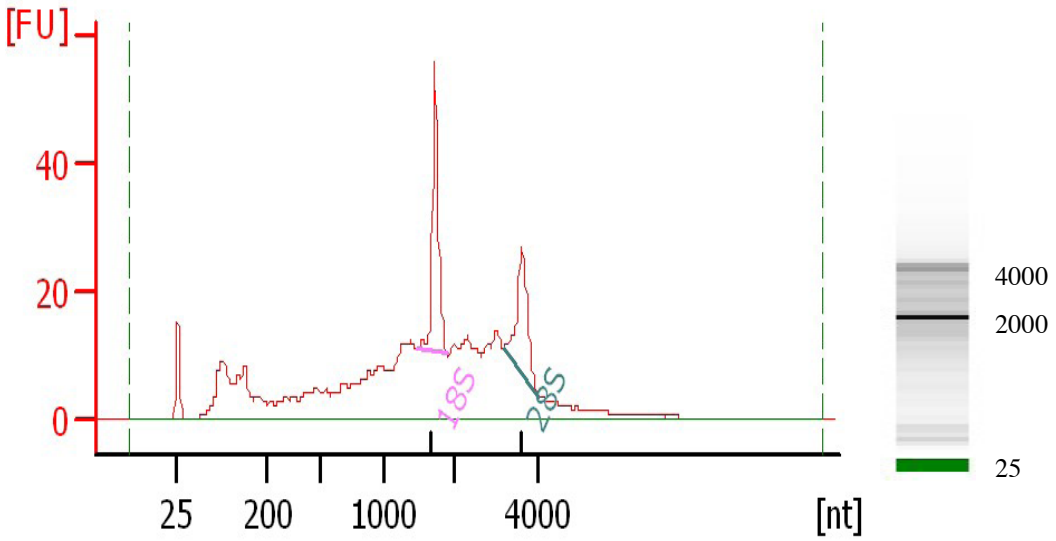


**Figure 4.1:** Representative electropherogram summary of a baboon endometriotic RNA extraction. Height threshold [FU] and ladder peaks [nt]. Adjacent gel output represents ladder peak markers. Measured RNA concentration = 234 ng/µl; rRNA Ratio [28s/18s] = 0.7 and RNA Integrity Number (RIN) = 6.0. Refer to Figure 3.1 for a summary of the ladder marker supplied by Agilent Technologies.

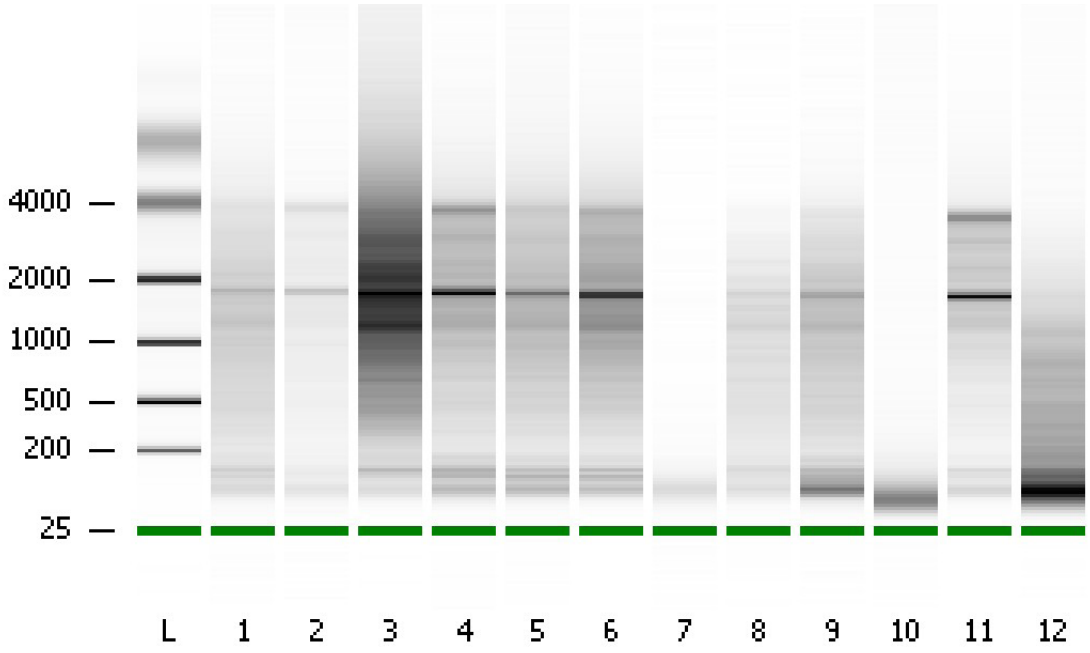


**Figure 4.2:** Representative gel output of a baboon RNA extraction whereby samples were unsuitable for further mRNA analysis. Lanes 1-10 and 12 were excluded in the study, as the RIN and quality were compromised. Ladder markers are represented on the y-axis, whilst the x-axis represents an individual baboon RNA sample from lyophilised tissues.

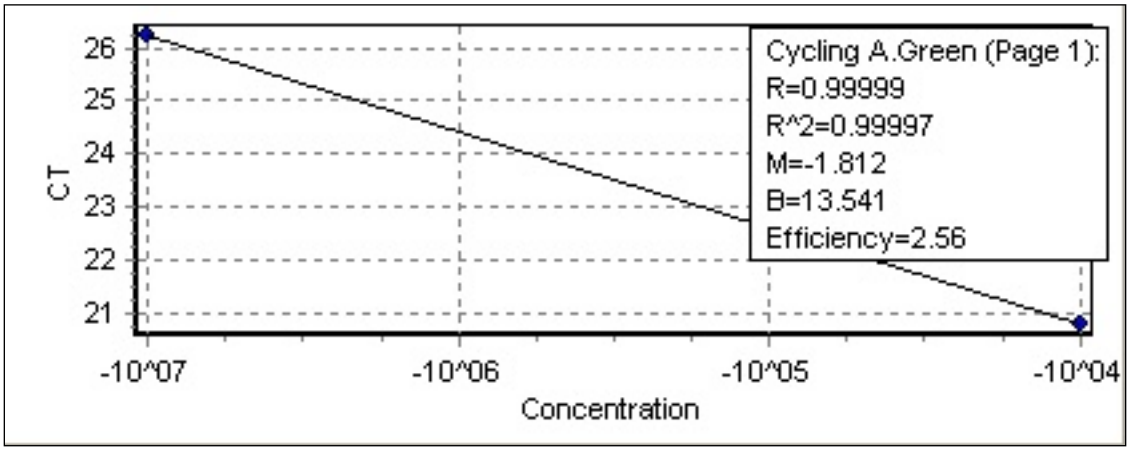


**Figure 4.3:** Representative standard curve for NFκβ in RNA extracted from baboons with endometriosis, normalized against 18S. y-axis represents the cycle threshold, whilst the x-axis represents the concentration of extracted baboon RNA sample.

## 4.4 Discussion

Similar to Section 3.4, a number of lyophilised tissue samples from baboons were collected but were excluded from the study as RNA concentrations and integrity were severely compromised. In some cases, RNA was not detected or only a faint 18S band was present.

Table 4.1 reveals that NFκβ is not present in ectopic endometriosis tissues of baboons. This mimics the result found in humans in Chapter 3. An investigation of eutopic endometrial tissues from baboons is also required to determine any fold change between the eutopic and ectopic endometrium but this was not possible due to low RNA quality obtained from lyophilised tissues.

The results in this study were verified using immunohistochemistry, so as to ascertain that NFκβ is potentially not the pathway involved in ectopic cell survival in endometriosis. The results for the baboon protein study can be seen in Chapters 5 and 6.