Effects of norethisterone acetate on the performance of female racing Greyhounds

Richard M. Payne [*]

School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington Campus, LE12 5RD, United Kingdom

ARTICLE INFO	ABSTRACT
	A comparison between the performance of spayed female racing Greyhounds and those suppressed with norethisterone acetate (NTA) was made. Previous work by the author has shown that the racing performance of spayed bitches is the same as that of entire bitches in anoestrus, i.e. spaying is just a permanent anoestrus. The aim was to assess any performance difference between suppression and anoestrus, and thus to determine the effect of norethisterone acetate on race performance. The study was designed as a retrospective case-control. Raceform data was obtained for female racing Greyhounds which had raced, and which were either spayed or suppressed with norethisterone acetate. Analysis showed that suppressed bitches run on average 0.049 to 0.061 s slower over 480 m (centralised models). Since endogenous progesterone (P4) has been linked with reduced race performance, it is logical that progesterone analogues like NTA should have a similar effect. It is likely that the depression in performance is dose-related, but not quantifiable with the current dataset.

1. Introduction

1.1. Study objectives

The aim of the study was to determine the effect on performance of norethisterone acetate (NTA) in UK racing Greyhound bitches by analysis of raceform data.

1.2. Background

For many decades female racing Greyhounds have been medicated to postpone or suppress their oestrous cycles. Suppression of oestrus is common, and results from a desire to maintain the bitches in steady work, rather than have a lengthy time off: the GBGB[1] Rules of Racing prohibit bitches from racing or trialling for 21 days after a season (oestrus) is reported. Anecdotal reports indicate that female Greyhounds show variations in their oestrous cycles, changing from biannual oestrus to annual or even anoestrus (no data), probably by a similar mechanism that causes changes in female human athletes (De Souza et al., 1991; Frankovich and Lebrun, 2000). Cyclicity is normally restored when the Greyhounds retire (usually by five years old). Previous work by the

author suggested that the layoff period should be nearer to 60–70 days (Payne, 2013a) in order to avoid the performance changes seen at 40–55 days post-oestrus. This performance change is presumed to be due to elevated levels of endogenous progesterone (P4). Another reason for suppression is to avoid the behavioural changes associated with false pregnancy (driven by prolactin), which can last for up to 90 days post-oestrus.

In the past, various medications have been used to suppress/postpone oestrus: notably testosterone esters like methyltestosterone (MTA) (Romagnoli, 2009) but avoiding the progesterone analogues (PA) such as megestrol acetate (MA) and medroxyprogesterone acetate (MPA) as it is well-known by Greyhound trainers that these analogues markedly depress performance. From 23rd March 2011 the GBGB, after discussion with the UK Veterinary Medicines Directorate,[2] allowed the use of the progesterone analogue norethisterone acetate (NTA), prescribed by veterinary surgeons under the cascade system. Following that, from 1st January 2013, methyltestosterone was banned in female racing Greyhounds (with a transition period to 4th March 2013). Trainers were encouraged to opt for spaying as a result of research by the author (Payne, 2013b), but the uptake of this option has been slow due to the obvious permanence of surgery. In Western Australia a permanent ban

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on anabolic androgenic steroids in racing Greyhounds was imposed on 1st January 2016. Under Australian rules ethyloestrenol is permitted, along with the progestagens and GnRH analogues, whilst no human preparations are permitted (Arnott, 2016).

The shift from a testosterone ester to a progestogen was to try to avoid both the side-effects and potential misuse of testosterone esters, viz.: clitoral enlargement and possible performance enhancement in bitches (Biddle et al., 2009). Current analytical techniques can distinguish between the various steroid hormones for integrity purposes (Biddle et al., 2013).

1.3. Action of progesterone analogues

Steroid hormones affect either the hypothalamus or pituitary gland. Progesterone acts on the pituitary gland to reduce the responsiveness to GnRH, decreases the amplitude of GnRH pulses and can block the effect of oestradiol on GnRH receptor expression. In doing so reproductive cyclicity is suppressed. Sensitivity to GnRH increases as progesterone levels fall rather than due to increases in oestradiol. Removal of progesterone does not cause an increase in GnRH receptors because GnRH itself is an important regulator of GnRH receptors. Hence progesterone affects pulsatility by reducing GnRH production which thus reduces GnRH receptor numbers i.e. progesterone does not inhibit GnRH receptor production directly. Progesterone also inhibits the production and secretion of LH and FSH (Nett et al., 2002), as well as rendering the uterine environment unfavourable for implantation (Jamin et al., 2003).

1.4. Manipulation of the oestrus cycle with progesterone analogues

Progesterone analogues such as MA, MPA and MTA block the production and release of GnRH. Administration of progesterone analogues in the presence of pre-existing disease will worsen the signs. PA should not be used for pseudopregnancy nor during oestrus or prolonged oestrus. Ideally medication should start in anoestrus. Likewise PA should not be used in pre-pubertal females (Romagnoli, 2006). In the 1970's Prole conducted a pilot study on the use of norethisterone in Greyhound bitches (Prole, 1974a) but it has only been recently that NTA has seen more widespread use in racing Greyhounds. Norethisterone has a licence for human use to treat various disorders, including endometriosis and dysmenorrhea, but also inhibits ovulation by suppressing the mid-cycle LH peak.

1.5. Side-effects of progesterone analogues

Analogues such as proligestone and MPA have side-effects in racing Greyhounds, most notably a reported drop in performance and weight gain, hence the original reason to use methyltestosterone. As far as performance animals are concerned, there are several consistent and fully reversible side-effects of progestogens, centred around weight gain and reduced exercise performance, effects which can be nulled by spaying (Payne, 2013a, 2013b). Norethisterone has oestrogenic activity in dogs (unlike MPA), so the performance loss may be less, given that bitches coming into season run faster (Payne, 2013a), which matches the rise in oestrogen (Edqvist et al., 1975). This is not proven to be a causative link, only temporally linked. The last analysis of the effects of NTA on racing Greyhounds was performed by Prole (1974b) indicating a need for up-to-date analysis.

2. Materials and methods

2.1. Data capture and management

Data for signalments, dates of oestrus or suppression together with racing performance was obtained from PA Sport with permission of the GBGB. This data was formatted in Microsoft Excel 2016[3] and then imported into Microsoft SQL Server Express 2014,[4] using Microsoft Access 2016[5] as the frontend. Data for the suppressed Greyhounds was restricted to races since 15th March 2013, since after that date only NTA was used as a suppressant. Data for spayed Greyhounds was the same as gathered and prepared for previous research (Payne, 2013b). Data extraction was via standard SQL code written against the MS Access database and exported into an Excel spreadsheet. Excel also served as the intermediary for import of raceform and signalment data out of SQL Server/Access into MLwiN 2.36 (Centre for Multilevel Modelling, University of Bristol[6]) for model generation.

2.2. Inclusion criteria

To be included in the dataset, Greyhounds had to be female, of any age, and to have raced in graded races under GBGB Rules of Racing. Within this data there was information on the reproductive state or date of spay/last oestrus.

2.3. Allocation to groups

The Greyhounds were allotted to one of two groups: (1) spayed or (2) suppressed. Use of spayed Greyhounds as a control set was justified on the basis that (a) spayed Greyhounds are hormonally inactive and (b) previous have shown that the performance of anoestrus Greyhounds is the same as that of spayed Greyhounds (Payne, 2013b). Using spayed Greyhound data allowed a more consistent control set than raceform data from entire bitches in anoestrus, as their true anoestrous period cannot be determined, whereas spayed bitches are in permanent anoestrus.

2.4. Outcome variable

The outcome variable was deemed to the individual Greyhounds' race times, expressed in seconds as the variable *IndTime*. The raceform for open race Greyhounds was excluded as this would create a multimembership model and thus introduce unnecessary complexity and error.

2.5. Power and sample size calculations

Statistical power calculations were performed in the GPower 3.1[7] and Optimal Design[8] programs, based on a 0.03 s difference in race times. The overall large n for the data overcame the problem of lower effective sample sizes in multilevel models, since a 95% power could be achieved by setting alpha at 0.1 and using a sample size of >1300 individual races for each group. This also produced equal Type I and Type II error rates. The actual dataset produced $n = 30,838$ repeat measures for the suppressed cohort, and 11,203 repeat measures for the spayed cohort.

2.6. Statistical analysis and modelling

Univariate analyses, correlations and scatter plots were performed

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using StatsDirect.[9] Multilevel modelling and model checking was performed within MLwiN 2.36.

The outcome measure was always individual race time (*IndTime*) in seconds. Each Greyhound had several races, representing repeated measures of *IndTime*. The first level within the model was set as repeated races, with Greyhound identity as the second level. Models were built in a forward stepwise manner using distance (metres) as the main predictor, with weight (kilograms) and age (days) as confounders based on previous experience with modelling such data (Payne, 2013a). Spayed versus suppressed was indicated via a dichotomous variable (0,1). Model checking was by plotting of standardized residuals against normal scores and plotting standardized residuals against fixed first part predictions.

3. Results

3.1. Performance of spayed Greyhounds – 250-800 m

A scatter plot of distance against time was produced, which showed a linear relationship with a very strong correlation. Fig. 1. The correlation for this relationship is $r = 0.958$, $r^2 = 0.918$, showing how consistent Greyhounds are over a wide variation of distances. The output of the multilevel model with random slope produced the following equation for race time (seconds):

$$IndTime_{ij} = 5.498(0.170) + 0.058(0.000)\text{Distance}_j - 0.146(0.006)\text{Kg}_j + e_{ij}$$

Equation 1: MLM for race time in seconds for spayed Greyhounds. Distance is seconds/m. The residual variance, $\sigma_e^2 = 0.843(0.013)$, $n = 8520$. Numbers in parenthesis are standard errors.

The independent variable Distance is expressed as seconds/m, hence 0.058 s/m equates to 17.24 m/s, which is within the expected speed (author's unpublished analyses).

3.2. Performance of suppressed Greyhounds – 250-800 m

A scatter plot of distance against time was produced, which showed a linear relationship with a very strong correlation, see Fig. 2. The correlation for this relationship was $r = 0.988$, $r^2 = 0.976$, again showing how consistent Greyhounds are over a wide variation of distances. The output of the multilevel model produced the following equation for race time (seconds):

$$IndTime_{ij} = 1.100(0.065) + 0.062(0.000)Distance_j - 0.043(0.002)\text{Kg}_j + e_{ij}$$

Equation 2: MLM for race time in seconds for suppressed Greyhounds. With residual variance of $\sigma_e^2 = 0.502(0.004)$, $n = 30,724$ with SEM in parentheses.

As before, 0.062 s/m equates to 16.12 m/s, which is within the expected speed (author's unpublished analyses).

3.3. Combined model spayed and suppressed performance – 250-800 m

As a backup for the individual models, a combined multilevel model was constructed with both datasets, giving the following equation:

$$IndTime_{ij} = 1.876(0.063) + 0.061(0.000)\text{Distance}_j - 0.065(0.002)\text{Kg}_j$$
$$+ 0.049(0.010)\text{Suppressed}_j + e_{ij}$$

Equation 3: MLM for race time for spayed and suppressed Greyhounds, where the variable "Suppressed" is a dichotomous indicator having 0 = spayed and 1 = suppressed. With residual variance of $\sigma_e^2 = 0.589(0.004)$, $n = 39,244$ with SEM in parentheses.

This model shows that suppressed bitches run on average 0.049 s

⁹ StatsDirect Ltd. StatsDirect statistical software. http://www.statsdirect.com. England: StatsDirect Ltd. 2013.

slower than spayed Greyhounds over all distances.

Results – distances restricted to 350–580 m.

In order to refine the analyses, the data for distances were restricted to 350–580 m for both groups, and the analyses repeated.

3.4. Performance of spayed Greyhounds – 350-580 m

A scatter plot of distance against time was produced which showed a linear relationship with a very strong correlation, as shown previously. The correlation for this relationship was $r = 0.916$, $r^2 = 0.840$, again showing remarkable consistency over the distances. The output of the multilevel model with random slope produced the following equation for race time (seconds):

$$IndTime_{ij} = 10.543(0.147) + 0.047(0.000)\text{distance}_j - 0.118(0.004)\text{Kg}_j + e_{ij}$$

Equation 4: MLM for race time in seconds for spayed Greyhounds. Distance is seconds/m. The residual variance, $\sigma_e^2 = 0.477(0.008)$, $n = 7957$ with SEM in parentheses.

3.5. Performance of suppressed Greyhounds – 350-580 m

A scatter plot of distance against time was produced which showed a linear relationship with a very strong correlation, as previously. The correlation for this relationship is $r = 0.970$, $r^2 = 0.941$. The output of the multilevel model with random slope produced the following equation for race time (seconds):

$$IndTime_{ij} = 2.492(0.123) + 0.059(0.000)\text{Distance}_j - 0.042(0.004)\text{Kg}_j + e_{ij}$$

Equation 5: MLM for race time, distanced restricted, suppressed Greyhounds. Residual variance $\sigma_e^2 = 0.415(0.006)$, $n = 8366$.

3.6. Combined model spayed and suppressed performance – 350-580 m

As before, a combined model was generated to compare spayed and suppressed performance, restricted distances.

$$IndTime_{ij} = 5.805(0.102) + 0.054(0.000)\text{Distance}_j - 0.083(0.003)\text{Kg}_j$$
$$+ 0.061(0.013)\text{suppressed}_j + e_{ij}$$

Equation 6: MLM for race time for spayed and suppressed Greyhounds, where the variable "suppressed" is a dichotomous indicator having 0 = spayed and 1 = suppressed. Restricted distances. Residual variance of $\sigma_e^2 = 0.515(0.006)$, $n = 16,323$, SEM in parentheses.

Suppressed bitches are on 0.048 to 0.074 s slower than spayed bitches, or approximately one length.

3.7. Overview of all models

Table 1 brings together the models generated above. The MLM results show that suppressed bitches run on average 0.049–0.061 s slower (MLMs eq.3 and eq.6). An alternative approach is to compare speeds (produced as seconds per metre by the *Distance* variable) between similar models. Over a wide range of distances, spayed Greyhounds are 0.004 s/m faster than suppressed (MLMs eq.1 versus eq.2); whilst in the restricted distance models, spayed Greyhounds are 0.012 s/m faster (MLMs eq.4 versus eq.5). Note here that these are model comparisons, because the intercepts are not fixed at zero: i.e. the linear models do not take account of the acceleration phase, hence the *Distance* variable describes an average speed, not the true maximum speed. By performing an example calculation, some idea of the actual race times can be constructed. By comparing the example times for MLMs eq.1 versus eq.2, and eq.4 versus eq.5, the estimated time differences suggest that suppressed bitches can be faster or slower than spayed bitches. This might suggest an error, but models are variable in their predictions and given that the other comparisons all show that suppressed bitches run slower,

Plot of IndTime v Distance
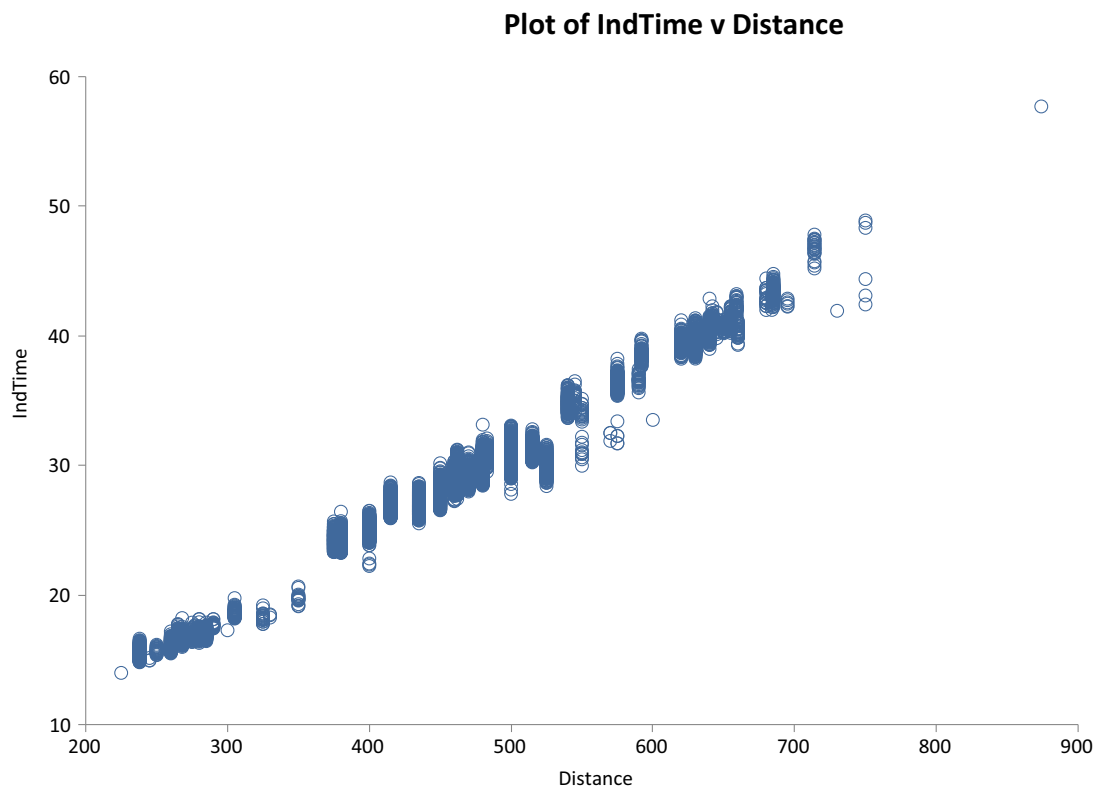
Fig. 1. Scatter plot of distance against race time, for spayed Greyhounds.
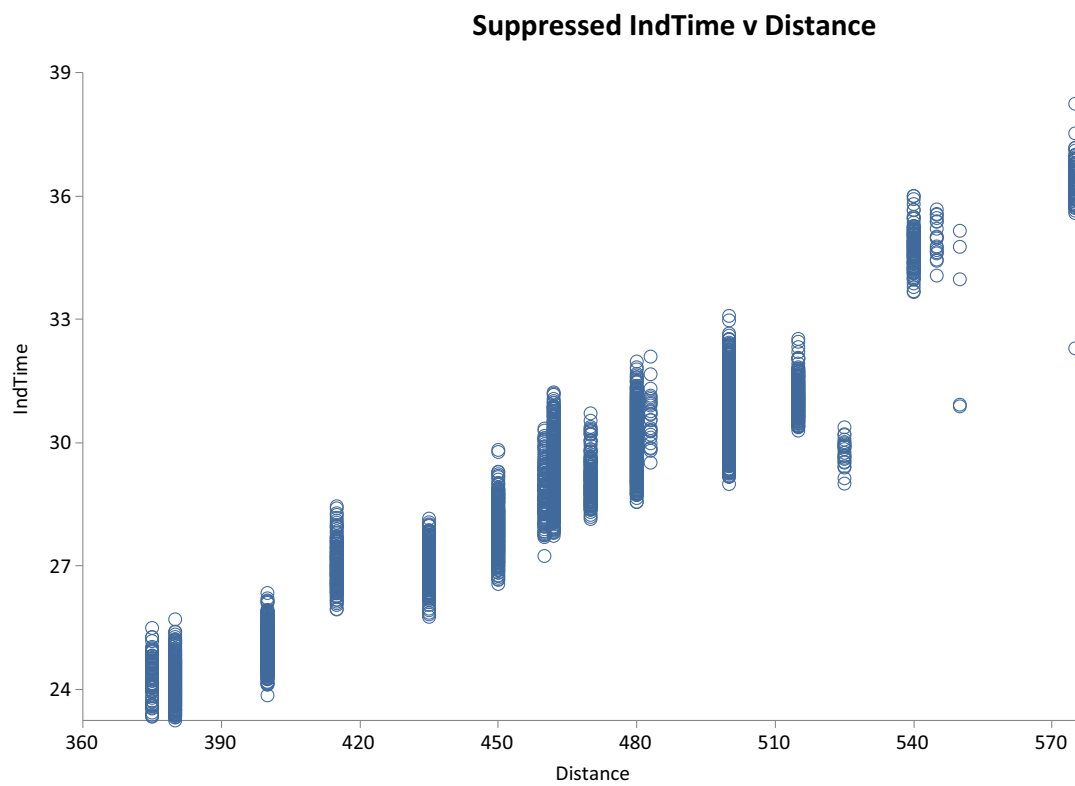
Suppressed IndTime v Distance

Fig. 2. Scatter plot of distance with race time for suppressed Greyhounds.

Table 1
Combined results from all MLM. Standard error of means within square parentheses. Standard error of the mean for *Distance* was always 0.000.

MLM type	IndTime (*sec*)	Distance (sec/m)	Weight (sec/Kg)	Suppressed time difference (sec)
Eq. 1: Spayed, 250–800 m	5.498 [0.170]	0.058	−0.146 [0.006]	n/a
Eq. 2: Suppressed, 250–800 m	1.100 [0.065]	0.062	−0.043 [0.002]	n/a
Eq. 3: Combined, 250–800 m	1.876 [0.063]	0.061	0.065 [0.002]	0.049 [0.010]
Eq. 4: Spayed, 350–580 m	10.453 [0.147]	0.047	−0.118 [0.004]	n/a
Eq. 5: Suppressed, 350–580 m	2.492 [0.123]	0.059	−0.042 [0.004]	n/a
Eq. 6: Combined, 350–580 m	5.805 [0.012]	0.054	−0.083 [0.003]	0.061 [0.013]

this should be the overall conclusion.

4. Discussion

The use of NTA does reduce the overall performance by a small but significant amount, equating to 0.049 to 0.061 s over 480 m, or about three-quarters to one dog length. These findings parallel that of the fall in performance in dioestrus as endogenous progesterone (P4) levels rise, as shown previously by the author (Payne, 2013a). Although no causal link was shown, it was reasonable to assume that the fall in performance was due to the dioestrus rise in endogenous P4. As such it is logical that progesterone analogues should also have a depressor effect on performance. The fall in performance with NTA is not as large as with endogenous P4, viz.: 0.049 to 0.061 s with NTA versus 0.031 to 0.733 s for P4 (P4 results from Payne (2013a). This fits with evidence-based knowledge that NTA does not appear to produce the marked depression in performance seen with other PAs. This may be due to the oestrogenic effects of NTA which offset the progestagenic effects to some degree.

This performance difference needs to be compared with the effect of weight: heavier Greyhounds run more quickly owing to higher muscle mass, and the effect of lower body weight is one order of magnitude greater than the effect of being suppressed. What must also be taken into account is the effect of the dose rate on performance, data for which is not available. Although the standard advised dose of NTA is 5 mg daily, variations from this do occur but it is not possible within the existing datasets to include animal-specific dose information. Therefore, the suppressed bitches are assumed to be adequately suppressed, whereas in fact they may only be partially suppressed. The fact that steroids have a dose-response relationship means that heavier bitches will have a lower dose and therefore have a lesser response both in terms of effective oestrus suppression and depression of performance i.e. there is an unquantified dose-related effect on performance which will be reflected in the race form. This may be the reason why trainers report varying depressive effects on performance, since effective suppression is associated with doses of 5 mg NTA per day, whereas 2.5 mg NTA per day is linked with less effective suppression but fewer side effects (author's data). It is not possible with the current dataset to quantify the magnitude of this problem so that dose rate and performance changes can be modelled, other than to say that there is a negative effect of NTA on performance.

5. Conclusions

Overall, the use of NTA does significantly detract from the performance of racing Greyhounds. The reduction in performance is to be expected from a progesterone analogue, but the reduction caused by NTA is much less than that of other PA, and less than that seen in naturally cycling bitches which may be racing after returning from seasonal layoff. It is proposed that the depression in performance is dose related.

Declaration of Competing Interest

The author expresses no conflicts of interest.

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