Stimulation of delayed puberty in heifers by using a PRID regime

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SUMMARY

The objective of the present study was to stimulate delayed puberty in heifers by using a PRID (a progesterone releasing intravaginal device, impregnated with 1.55 g of progesterone and 10 mg of oestradiol benzoate) regime. Prior to the study, individual ages (20 to 30 months) and body condition scores, BCS (2.25 to 3.5 units; 1-5 scale with 0.25 intervals) were recorded. For each of 33 heifers (29 Brown Swiss, 4 Holstein) with delayed oestrus (smaller ovaries with no cyclic structures), a PRID apparatus was then inserted for 12 days. Following the withdrawals, fixed-time intracervical inseminations (AI) were performed at 48 and 72h. Blood samples were collected by jugular venipuncture on the 21st day of AI for determining plasma progesterone (P4) concentrations by EIA. Rectal palpations were also performed on the 60th day to confirm pregnancies. Results showed that the vast majority of heifers (93.9 per cent. 31/33) showed cyclicity (P4 greater than 1.0 ng per ml) and a total of 54.6 per cent (18/33) pregnancy rate was obtained by PRID stimulation. A close relationship (R²=0.718, P lower than 0.001) was found between the P4 (varying from 0.63 to 5.86 ng per ml) and pregnancies. However, neither the age nor BCS in situ did significantly affect the cyclicity or pregnancy rates.

Findings suggest that PRID can be used effectively to stimulate puberty (cyclicity) and that the early P4 determination (on the 21st day post-insuination) is highly indicative of the induced cyclicity (P4 greater than 1.0 ng/ml), allowing for the establishment of subsequent pregnancy in heifers with delayed puberty.

Keywords: Heifer, puberty, PRID, progesterone, pregnancy.

Mots clés : Génisse, puberté, PRID, progestérone, gestation.

Introduction

In females, puberty is characterized by appearance of the first signs of oestrus and it depends mainly upon breed, climate and management. It occurs by an increased GnRH release thus allowing gonadotropins to be released leading to stimulation of the ovaries.

In cattle breeding, delayed oestrus (thus late pregnancy) leads to major economic losses. Additionally, the frequency of problems related to monitoring oestrus signs or high incidence of suboestrus cases can increase the losses due mainly to improper herd management strategies.

It is known that although both endocrinological and neuroendocrinological events play a major role in the commencement of puberty, the actual nature of sexual maturation has not yet been fully understood [1, 9]. However, according to the 'gonadostat theory' [26], it was claimed that the negative feedback effect of oestradiol lowers the sensitivity of hypothalamo-hypophysial centre (axis) regulating gonadotropin secretion needed to the commencement of puberty. By a lower negative feedback effect of steroids, gonadotropin secretion increases allowing follicular maturation and ovulation to take place [9, 35].

Available data indicate that many individual components of the reproductive endocrine system in the heifer are operational before the oestrous cycles are initiated. For example, prepubertal heifers respond to exogenous gonadotropin-releasing hormone [4] and to the positive feedback effects of oestradiol [28] with LH surges similar to or greater than those elicited by these stimulations in mature cattle [9].

Therefore, the objective of the present study was to stimulate delayed puberty (cyclicity) in heifers that have been kept under similar management (housing and feeding) conditions by using a PRID regime.
Materials and Methods

EXPERIMENTAL MATERIAL USED

In the present study, a total of 33 heifers (29 Brown Swiss, 4 Holstein breed) aged 20-30 months old and having a mean BCS of 3.0, as ranged from 2.25 to 3.5 (1-emaciated to 5-obese scale with intervals of 0.25) [33] were used. The animals used in this study had no previous history of oestrus signs.

Prior to the study, rectal palpations were performed on two occasions (10 days apart) to examine whether any pathological evidence on ovaries or uterus in heifers. In the absence of any pathological disorder, it was also confirmed that there was no pathological finding within the vagina during further examination by a speculum. Considering other clinically healthy heifers (showing cyclic activity) within the same herd, the ovaries of animals without cyclic activity were smaller and had no cyclic structures. All these initial findings indicated that these heifers concerned had not yet reached the puberty (delayed puberty).

MANAGEMENT

Heifers were kept under the same management conditions (housing and feeding) in one of the farms in Erzurum, TURKEY. The study was conducted in summer and the animals having free access to outdoor shelters were fed by medium quality dried grass hay with water *ad libitum*.

PRID ADMINISTRATION AND AIS

Prior to the PRID administration, the vulva was dry cleaned with a tissue paper. The apparatus (each impregnated with 1.55 g of P4 and 10 mg of oestradiol benzoate) was then inserted dorso-cranially into the vagina via its special applicator for 12 days. Following the PRID withdrawals, no observation was made for the signs of expected oestrus. Instead, fixed-time intra-cervical AIs were performed using fertility-proven frozen-thawed semen (Lalahan Livestock Central Research Institute, Ankara-TURKEY) in 0.25 cc straws (20x10^6 total sperm with a minimum of 50% post-thawing motility per dose) by the same trained staff at 48 and 72h.

PROGESTERONE ANALYSIS

On the 21st day post-inseminations, blood samples (10 ml) were collected into vacutainer (with EDTA) tubes by venipuncture (jugular vein) for determining plasma P4 concentrations by EIA (Enzyme immunoassay). All the samples collected were centrifuged at 2,000 x g for 15 min and plasma were then kept at -18°C until analysis. Plasma samples were analyzed using a double-antibody EIA technique for determination of P4 as described by PRAKASH et al. [24]. All the assays were carried out in 96 well microtiter plates (Nunc-Immunoplate, Cat. No. 439454, Brand Products, Denmark) and standards, samples and controls were studied in duplicate. The intensity of color was measured at 450 nm with an 8-channel microtitration plate photometer (Tecan, Spectra III, A 5082, Austria) and the results were evaluated using EasyWin Kinetics software supplied by Tecan. The sensitivities of assays were 0.5 ng/ml. Intra-assay coefficients of variations were 7% for the test.

CYCLICITY DETERMINATION

The induced cyclicity was determined by considering the concentration of blood P4. The animals were considered ‘cyclic’ when the plasma P4 values were equal or higher than 1 ng/ml while others with lower concentrations were considered as “non-cyclic” [30].

PREGNANCY DIAGNOSIS

Rectal palpations were performed for the actual pregnancy diagnosis.

EFFECTS OF AGE AND BCS UPON THE CYCLICITY AND PREGNANCY RATES

Firstly, the overall effects of age and BCS upon both the cyclicity and pregnancy rates were considered. A further analysis was also made for possible effects of both the mature age and the conditioned status by considering the two arbitrary age (equal and/or younger or older than 24 months) and BCS (equal and/or lower or higher than 3.0 units) categories.

STATISTICAL ANALYSIS

Data (represented as mean ± SEM) were analysed by Pearson’s correlation, Chi-square and regression analysis using MINITAB statistical software programme [21]. Differences were considered significant when P<0.05.

Results

Overall results showed that virtually all the heifers (93.9%, 31/33) with delayed puberty had cyclicity (P4>1.0 ng/ml) and a total of 54.6% (18/33) pregnancy rate (by rectal palpation) was obtained by PRID administration (Table I).

Considering the age (20 to 30 months) of heifers, there were no significant effects of age *in situ* upon the overall cyclicity or pregnancy rates. However, there was a relatively significant (P≤0.057) relationship between the age of heifers and their cyclicity due mainly to the two of the old-aged (≥29 months) individuals (ID no 21 and 22), showing no cyclicity. Broadly, nevertheless, older heifers (>24 months) tended (P=0.062) to have higher pregnancy rates (65.22%±10.20) than those (30.00%±15.30) in younger ones (≤24 months).

Considering the BCS (2.25 to 3.25 units) of heifers, there were also no significant effects of BCS in situ upon the overall cyclicity or pregnancy rates. However, it appeared that a higher BCS (> 3.0 units) tended to result in higher pregnancy...
rates (66.67%) as compared with those (47.62%) with lower BCS (≤3.0 units) (P=0.290).

Finally, a close relationship (R²=0.718, P<0.001) was found between the plasma P₄ concentrations and pregnancy rates such that the higher concentrations (ranged from 3.42 to 5.86 ng/ml) were usually associated with a subsequent pregnancy (94.74%) in cyclic animals (n=31), except for one individual (ID no 17) with 4.56 ng/ml P₄.

**Discussion and Conclusion**

In livestock animals, numerous studies have been conducted upon shortening the age of puberty [3, 9]. Stimulation of delayed puberty is highly important for improving the reproductive performance. For this aim, some techniques including the administration of ovarian steroids such as the P₄ or progestagens have been used for advancing the pubertal period [1, 29]. Additionally, progestagens are particularly favourable for the initiation of oestrus and ovulation in prepubertal heifers and anoestrous cows [1, 11, 13, 16, 35].

Progesterone and oestradiol contained within the PRID are absorbed by the vaginal mucosa. Oestradiol reaches its high levels at the beginning of administration while the P₄ reaches up to the levels as seen during the luteal phase of cyclic animals throughout the administration period. The former elevation of oestradiol is also accompanied by an endogenous increase. Oestradiol has a negative feedback effect on the FSH release while the P₄ has the same effect on LH, as both collectively synchronising the possible follicular wave [10].

**Table 1**: Individual details (breed, age, BCS, P₄ concentration and rectal palpation) of heifers with delayed puberty used in the study

<table>
<thead>
<tr>
<th>Animal ID</th>
<th>Breed</th>
<th>Age (mo.)</th>
<th>BCS (1-5)</th>
<th>P₄ (21st day post-AI)</th>
<th>Cyclicity (P₄&gt;1.0ng/ml)</th>
<th>Rectal palpation (60th day post-AI)</th>
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<tbody>
<tr>
<td>1</td>
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<td>25</td>
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<td>4.48</td>
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<td>5.86</td>
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<tr>
<td>4</td>
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<td>3.47</td>
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</tr>
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<td>3.25</td>
<td>3.42</td>
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</tr>
<tr>
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<td>Non-pregnant</td>
</tr>
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<td>0.63</td>
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<td>Non-pregnant</td>
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<td>1.31</td>
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</tr>
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<td>1.22</td>
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<tr>
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<td>3.0</td>
<td>1.74</td>
<td>Cyclic</td>
<td>Non-pregnant</td>
</tr>
<tr>
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<td>1.16</td>
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<td>2.75</td>
<td>1.51</td>
<td>Cyclic</td>
<td>Non-pregnant</td>
</tr>
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</table>
As a result, these lead to the storage of gonadotropins and their ultimate release by the pituitary. Following the PRID withdrawal, the source of exogenous P₄ disappears, leading to a LH wave and thus ovulation. This is essential for a normal follicular development following the withdrawal. Additionally, DAY et al. [9] reported that the negative feedback effect of oestradiol on the LH is more apparent in pre-pubertal heifers than in pubertal ones. In recent studies, the P₄ was also proved to be a reliable alternative for stimulation of inactive ovaries [13, 35].

The effectiveness of P₄ administrations for the initiation of puberty in heifers is related closely to age, breed, nutrition (BCS) and the degree of follicular development prior to the administration [5, 23]. Heifers used herein were 20 to 30 months of age and had the mean BCS of approximately 3.0 (ranged from 2.25 to 3.5) units without any significant effects in situ upon the cyclicity or pregnancy rates. Nevertheless, there was a relatively significantly (P≤0.057) adverse relationship between the age and cyclicity, due mainly to the effects of the two old-aged (≥29 months) heifers (ID no 21 and 22), as both showing no cyclicity. In a broader sense, however, the pregnancy rates tended (P=0.062) to be higher (65.22%±10.20) in older (≥24 months) heifers as compared to those (30.00%±15.30) in younger (<24 months) ones. On the other hand, it appeared that a higher BCS (>3.0 units) tended to result in higher (P=0.290) pregnancy rates (66.67%) as compared to those (47.62%) with lower BCS (<3.0 units). Given these results, it appeared that both the mature age (>24 months) [5] and nutritional (conditioned) status (BCS>3.0 units) might have some degree of favourable effects upon the pregnancy rates [see 23 for further details]. However, as the scope of the study was to stimulate delayed puberty by using PRID only, no further analysis was made on the particular effects of age or BCS concerned.

ANDERSON et al. [1] reported that progestagens stimulate puberty very effectively. In the present study, virtually all the heifers (93.94%) had cyclicity (P₄>1ng/ml) following the stimulation by PRID, clearly showing its high effectiveness as a drug of choice. HALL et al. [13] suggested that greater results can be obtained depending on the maturity (age) of animals receiving the P₄ administrations. As discussed earlier, this was also partly the case herein such that the heifers with mature age (older than 24 months) tended (P=0.062) to have a higher pregnancy rates (65.22%) as compared to those of younger ones. On the other hand, however the former researchers considered that the effectiveness of P₄ in the stimulation of puberty is related mainly to the functionality of endocrinological system regulating the LH release rather than to the age of female.

Furthermore, ANDERSON et al. [1] reported that only the 67% of animals among older heifers initially stimulated with progestagens reached puberty. Additionally, FIKE et al. [11] observed that the luteal function was stimulated in 55% of heifers following progestagen stimulation. Likewise, UNAL et al. [32] achieved a 50% oestrus and pregnancy rate in heifers with no signs of puberty by using a PRID regime. In some studies conducted upon the stimulation of puberty, FORLAND et al. [12] observed a 60% pregnancy rate while ZULU et al. [35] obtained a much lower rate of 28.6%. In the present study, the pregnancy rate was 54.45%, clearly indicating the pubertal maturity of those heifers concerned. The present results are in parallel with those of the majority of studies.

In addition, ANDERSON et al. [1] reported that although the P₄ administration did not affect the follicular development, the uterus became markedly enlarged following the implant withdrawal in heifers. They considered that this rapid enlargement during the pubertal period could be originated from the increase in oestradiol level related to follicular development. It was considered that this mechanism might also be one of the main underlying reasons of pregnancies obtained in more than half of the heifers used herein.

In the present study, there was a close relationship (P<0.001) between the P₄ levels and rectal palpation findings (pregnancy rates) on the 60th day post-insemination. However, one of the animals (ID= 17) was found to be non-pregnant although its P₄ value on the 21st day was quite high (4.56 ng/ml). This might have been due firstly to earlier regression of corpus luteum in that heifer probably returned to oestrus earlier and thus having an ongoing (subsequent) corpus luteum. Indeed, premature luteolysis shortening the normal length of bovine oestrous cycle is a common phenomenon following the first ovulation in puberty or immediately after calving [34]. The second reason would be that given the high analytical P₄ result, the heifer might falsely be considered pregnant at an early stage of pregnancy that could be lost afterwards due to the luteal deficiency or embryonic death [25]. Indeed, the luteal deficiency during the first 3 weeks of pregnancy has been hypothesized as a cause of pregnancy failure [6, 20, 27]. On the other hand, a single blood sample collected at 21 days after the AI may not be enough to determine, with certainty, the conceptus or to detect the heifers returned to oestrus. It means that the heifers with lower P₄ on that day could either be in anoestrus or in oestrus. If the animals are in oestrus (probably not too many heifers) they could be misinterpreted as non-responders to PRID stimulation. Thus, it also causes misinterpretation to predict the pregnancies with one blood sample. Based on this breeding schedule and the present experimental design (i.e. with no control group), the induced cyclicity rate rather than the pregnancy should be considered as the main outcome (or success) of the study because blood P₄ could be higher in most of the heifers if puberty has already been induced.

In the present study, there was no proper control group to compare the effectiveness of PRID administration to stimulate delayed puberty. This major deficiency in the experimental design would clearly make difficult to conclude as the proper choice of PRID for the stimulation of puberty. However, the farm management strategies and economical reasons did not allow having such group of control animals. Nevertheless, given the very high cyclicity (93.94%) and conventionally acceptable pregnancy rates (54.55%) obtained by using PRID only, its recommendation could easily be made for overcoming pubertal problems of heifers intended for breeding.

Overall, it was observed that PRID administration had a major contribution towards the sexual maturation of heifers with delayed puberty. It was considered that, the time (profitability) losses arising from the problems related to monitoring oestrus and/or subclinical oestrus may be minimised by such stimulations. Furthermore, it is suggested that in heifers
assumed physiologically to be at a mature age, the actual puberty may be initiated at an earlier age by some radical attempts to minimise the problems related to management strategies (especially feeding) of herds and thus a higher pregnancy rates may become more achievable by hormonal (mainly the P₄) administrations.

In conclusion, the present findings suggest that PRID can be used effectively to stimulate puberty and that the early P₄ determination (on the 21st day post insemination) is highly indicative of the induced cyclicity (P₄>1ng/ml) allowing for the establishment of subsequent pregnancy in heifers with delayed puberty.

References