Introduction

Artificial insemination (AI) in rabbit farms has become a common practice in numerous countries. According to Gogol (2009) this reproductive technology enables the development of a new cycled production system and a better organization of farms. One of the most important aspects of AI is the possibility of inseminating female rabbits regardless of their oestrus phase. The improvement and homogenization of reproductive performance on farms are conditioned by the use of methods enabling the induction and synchronization of oestrus including hormonal treatments or non-hormonal alternatives. Hormonal treatments have been widely used in recent years. With these treatments, different types and dosages of hormones are administered before insemination. The current management systems might be further improved, for example, by reducing the administration of exogenous substances or by simplifying the application of those substances which, at least at the moment, cannot be omitted because the AI requires the use of gonadotrophic hormones (GnRH) (Quintela et al., 2001). In rabbit does, ovulation does not occur spontaneously, but it has to be induced through a neurohormonal reflex, which is initiated during mating: hence, when using AI in the absence of a male, ovulation occurs by artificial methods (Hafez, 1993). Induction of ovulation requires the use of an intramuscular (IM) injection with GnRH or its synthetic analogues (Battaglini et al., 1982). Gonadorelin or buserelin have been shown to induce ovulation in rabbit does with results similar to those obtained by natural mating (Theau-Clément et al., 1990). Oestrus synchronization by means of equine chorionic gonadotropin (eCG) injection two or three days before insemination has become very common in industrial management, as this practice generally improves reproductive performance and it is simple to use (Bourdillon et al., 1992; Angeli et al., 1990; Gogol, 2004). Equine chorionic gonadotropin is a glycoprotein extracted from the serum of pregnant mares and has a hormonal follicle stimulating and luteinizing action. Several authors have suggested using prostaglandin (PG) F2α (PGF2α) injection by oral administration with sunflower oil (Sun) (rich in omega 6) or linseed oil (Lin) (rich in omega 3) on reproductive and productive performance. Group 1 was injected with 20 U of equine chorionic gonadotropin (eCG), 54 h before artificial insemination (AI) and used as reference group. Group 2 was injected with 20 U of eCG+0.5 mg of PGF2α, 54 h before AI. Group 3 was orally given 3 mL of Sun/doe/day, for seven consecutive days before AI+20 U of eCG, 54 h before AI. Group 4 was treated like Group 3 except that the oil was Lin. Aged does treated with eCG+Sun had elevated blood 17β estradiol concentration (P≤0.01) accompanied with a decrease of progesterone concentrations compared to the other experimental groups. Contrarily, no significant differences were found between eCG+Lin and eCG+PGF2α treatments on the previous two hormones. Likewise, aged does treated with eCG+Sun and eCG+Lin were statistically similar to those injected with ECG+synthetic PGF2α on blood prostaglandin profile, but still significantly higher than the control group. Treatment with eCG+Sun increased the percentage of fertile does (P≤0.01) and the litter size at birth compared to the other experimental groups. In conclusion, replacement of the PGF2α injection by oral administration of Sun or Lin to aged does improved sexual hormone synthesis and secretion, litter size and bunny body weight at birth.
Johnston (1990) demonstrated that the trienoic PG have lower biological activity than the dienoic PG and this may have direct effect on aspects of fertility. Another study of Mattos et al. (2000) showed that feeding a diet rich in linoleic acid could contribute to increasing secretion of PGF2α, compared with feeding a diet rich in linolenic acid.

Dairy cows fed diets enriched in PUFA had increased dominant follicle diameters compared to the cows fed a diet enriched with monounsaturated fatty acids (Bilby et al., 2006). Smits (2010) reported that the supplementation of fish oil (rich with omega 3 fatty acids) improved oocyte quality leading to enhanced blastocyst development, and further maturation of fish oil (rich with omega 3 fatty acids) increased dominant follicle diameters compared to a diet rich in linolenic acid.

Uterine endometrium secretes PGF2α, which induces an irreversible degeneration of CL, characterized by a dramatic drop in progesterone (P4) concentrations in the blood (Petit et al., 2002). The follicular phase begins after luteolysis and ends at ovulation. Gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH), released from the anterior pituitary, stimulate antral follicles to produce estradiol (E2).

This study was conducted to determine the effect of substituting the injection of PGF2α by oral administration of consecutive dosages of Sun and Lin on reproductive performance of aged rabbit does.

Materials and methods

The present study was carried out during winter season from October to April 2013-2014 (temperature ranged from 27 to 19°C, humidity 54 to 43% and light period 14 h light:10 h dark). Sixty does at 18-24 months of age with 304±±58.9 g average body weight, which had low conception rate and repeated refuse of mating with males were used in this study. Rabbits were randomly divided into four equal treatment groups of 15 rabbits each. Rabbits were housed in a naturally ventilated building and kept in individual galvanized wire cages with an internal nest-box, provided with feeders and automatic stainless steel nipple drinkers. All rabbits were fed ad libitum with a pellet ration containing 17.5% crude protein, 14% crude fibre and 2600 kcal/kg diet (Table 1).

The experimental groups were done as follows: Group 1 was injected with 20 U of eCG IM (Folligon; Intervet, Kempton Park, South Africa) 54 h before AI and used as a control. Group 2 was injected with 20 U of eCG IM (Folligon; Intervet)+0.5 mg Dinoprost, a synthetic analogue of PGF2α (Dinolytic; Pharmacia, Puurs, Belgium), 54 h before AI. Group 3 was orally administered with 3 mL of Sun/doe/day, for seven consecutive days prior to AI+20 U of eCG IM, 54 h before AI. Group 4 was orally administered with 3 mL of Lin/doe/day, for seven consecutive days prior to AI+20 U of eCG IM, 54 h before AI. Polysaturated fatty acid composition of Sun and Lin is presented in Table 2.

After 54 h of injection eCG IM and PGF2α, does were inseminated using a heterospermic pool diluted 3 times (1 semen:3 dilution extender). A pool of semen was collected from bucks of proven fertility was used for AI, which performed by depositing 0.5 mL of fresh diluting semen deeply in the vagina by sterile catheter. Semen was diluted with extender stored at room temperature (at 20°C) and used within 4 h of collection (semen ejaculates were individually evaluated microscopically and the ejaculates which showed active progressive motility percentages (over 70%) were pooled and used. Semen was added to Tris-buffer extender, so that the dilution rate was 1semen:3extender. The final concentration rate was 80-100 10 spermatozoa/mL. The insemination was immediately followed by the administration of 0.8 μg of buserelin IM (0.2 mL Receptal; Hoechst, Frankfurt, Germany) to induce ovulation.

Sexual receptivity of does was tested in the presence of a vasectomy buck as described by the International Rabbit Reproduction Group (2005). Fertility rate (number of parturitions/number of inseminations×100), litter size (total number of born) and bunny body weight at birth were recorded. Before AI, blood samples were collected from each doe immediately before AI and centrifuged at 3500 rpm for 20 min to obtain serum, which was stored at -20°C for later analysis. Serum samples were used for determining the concentrations of 17-β E2 (the Estradiol ELISA Test Kit has a sensitivity of 6.5 pg/mL) and P4 (the Progesterone ELISA Test Kit has a sensitivity of 0.105 ng/mL) by Immunoassay Technique Elisa Kits (Fortress Diagnostics Ltd, Antrim, UK), and PG concentrations (PGF2α) were measured using a PG ELISA kit (the Sensitivity: 50% B/Be:52 pg/mL) (Cayman Chemicals, Ann Arbor, MI, USA).

Statistical analysis

Data were statistically analyzed using one

| Table 1. Proximate analysis of pelleted basal diet. |
|---------------------------------------------------|
| Ingredients                                         |
|                                | Values | Calculated chemical analysis1 | Values |
| Bersseem hay                      | 30.0   | Crude protein                   | 17.5   |
| Barley grain                      | 21.0   | Crude fibre                     | 14.0   |
| Yellow corn                       | 5.0    | Ether extract                    | 2.7    |
| Wheat bran                        | 21.1   | Nitrogen free extract           | 56.4   |
| Soybean meal                      | 17.5   | Digestible energy, kcal/kg      | 2600   |
| Molasses                          | 3.0    | Folic acid, mg/kg               | 6.50   |
| CaCl2                             | 1.5    | Selenium, mg/kg                 | 0.20   |
| NaCl                              | 0.4    |                                  |        |
| Vitamin and mineral mix°          | 0.3    |                                  |        |
| DL-methionine                     | 0.2    |                                  |        |

1The vitamin and mineral premix/kg contained the following U/g for vitamins or minerals (rabbit premix produced by Holland Feed International Co., Heteren, The Netherlands): vitamin A, 4,000,000; vitamin D3, 5,000,000; vitamin E, 16.7 g; vitamin K, 0.67 g; vitamin B2, 2 g; vitamin B6, 0.67 g; vitamin B12, 0.004 g; vitamin B5, 16.7 g; pantothentic acid, 0.67 g; biotine, 0.07 g; folic acid, 1.67 g; choline chloride, 600 g; Zn, 23.3 g; Mn, 10 g; Fe, 25 g; Cu, 1.67 g; I, 0.25 g; Se, 0.05 g; Mg, 133.4 g. 2Based on National Research Council (1977). Values are expressed as percentage on a dry matter basis, unless otherwise stated.

| Table 2. Polyunsaturated fatty acid composition of sunflower and linseed oil. |
|-----------------------------------------------|
| Fatty acids, %                          |
|                                | 16:0 Palmitic | 18:0 Stearic | 18:1 Oleic | 18:2 Linoleic | 18:3 Linolenic |
| Sunflower oil                     | 6.0           | 4.6          | 17.8       | 69.2          | 0.1           |
| Linseed oil                       | 6.58          | 4.43         | 18.51      | 72.75         | 53.21         |

Polyunsaturated fatty acid composition of sunflower and linseed oil are based on references by Jasso de Rodriguez et al. (2002) and Pupa et al. (2012), respectively.
way ANOVA of SAS® (SAS, 1996). Differences among treatment means were separated by Duncan’s (1955) multiple range test.

**Results and discussion**

**Serum sexual hormones concentration**

Data of serum 17-β E\textsubscript{2}, P\textsubscript{4} and PG concentration of does with low conception rates and repeated refusal of males, treated with Sun or Lin as a source of PUFA for 7 consecutive days before AI instead of injecting with PGF\textsubscript{2α}, are illustrated in Table 3. Data revealed that 17-β E\textsubscript{2} recorded the highest (P≤0.01) value in rabbit does treated with eCG+Sun than ECG+Lin, eCG+PGF\textsubscript{2α} or the control group and the means were 115.8, 52.2, 40.1% above the control, respectively. On the other hand, rabbit does given eCG+Lin had 17-β E\textsubscript{2} level similar to that of does injected with eCG+PGF\textsubscript{2α}, which was still significantly (P≤0.01) higher than the control.

Serum P\textsubscript{4} concentration showed a significant decrease in all treated groups (eCG+Sun, eCG+Lin and eCG+PGF\textsubscript{2α}) compared to the control (eCG) and this decrease was -58.2, -41.7 and -43.8% less than the control value, respectively. In the meantime, does orally administered with Sun showed a significant decrease (P≤0.01) in serum P\textsubscript{4} level than the eCG+Lin or eCG+PGF\textsubscript{2α} groups, while, the last two treatment groups had a similar serum P\textsubscript{4} levels.

Injecting rabbits with PG 54 h before AI resulted in increased serum PG (P≤0.01) compared to the control group that was injected with eCG only and this increase was 24.9% above the control level. Giving rabbit does Sun or Lin for 7 consecutive days before AI had the same effect on increasing serum PG level as PGF\textsubscript{2α}, and this increase was significant (P≤0.01) compared to the control group (24.4 and 19.8% above the control value for Sun and Lin, respectively).

From previous data we can sum up that treated rabbit does with Sun or Lin for 7 consecutive days before AI as a source of PUFA boosted PG secretion in blood to rich approximately the level of that treated with the synthetic PGF\textsubscript{2α} analogues. This is due to the fact that Sun is a source of linoleic acid (66% of total fatty acids), which is an omega 6, whereas Lin is rich in linolenic acid (56% of the total fatty acids), which is an omega 3. Linoleic acid (C18:2n-6) is converted into arachidonic acid (C20:4n-6), which is the precursor of the dienoic (2-series) PG, such as PGF\textsubscript{2α}. Also, omega 3 in Lin (α-linolenic acid; C18:3n-3) is converted into eicosapentaenoic acid (C20:5n-3), which is the precursor of the trienoic (3-series) PG, such as PGF\textsubscript{3α}. Essential fatty acids include two series of PUFA that are involved in the maintenance of membrane function and are precursors for PG, in turn precursor of PG E1 and F1, and for the arachidonic acid (C20: 4 n-6), in turn precursor of PG E2 and F2 (Mead and Fulco, 1976). The PG is synthesized in the cell from the essential fatty acids (Laneuville, 2003) and both n-6 and n-3 fatty acids are stored in cell membranes and as substrates for the production of eicosanoids, such as PG (Calder, 2007). In addition, the high proportions of n-6 fatty acids (61% of total fatty acids for the Sun seed diet) could increase the secretion of series 2 PGs in blood (Petit et al., 2004). The PGF\textsubscript{2α} is implicated in reproductive functions such as ovulation, luteolysis and parturition. Actions of PGF\textsubscript{2α} are mediated by the PG F receptor (Sugimoto et al., 1994).

Increased blood PG level in Sun and Lin treated groups resulted in decreased blood P\textsubscript{4} level through its impact on the main function of CL, in the form of the P\textsubscript{4} hormone secretion causing a more complete regression of CL. Previous studies reported that uterine endometrium secretes PGF\textsubscript{2α}, which induces an irreversible degeneration of CL, characterized by a dramatic drop in P\textsubscript{4} concentrations in the blood (Petit et al., 2002). According to Kehl and Carlson (1981), 13, 14-dihydro-PGF\textsubscript{2α} caused premature luteal regression in rabbits. Watkins and Moore (1987) suggested that PG metabolites (PGMs) cause the release of oxytocin from the CL and lead to continuation of luteolytic activity. In the same context, PGF\textsubscript{2α}...
has been shown to possess luteolytic properties in rabbits (Abel et al., 1973; Ubilla et al., 1988; Rebollar et al., 1992) and to induce a fall in P4 concentration when it is administered on the last days of pregnancy (Ubilla et al., 1988). The precocious fall in P4 levels during the last days of pregnancy after treatment with PGF2α, can also exert an improvement of follicular growth (Ubilla et al., 1988) and, therefore, an earlier fall of the inhibitory effect of P4 on gonadotropin release (Battaglini et al., 1982). The results of Robinson et al. (2002) demonstrated that feeding cows with a Lin-rich diet (linoleic acid, C18:2n-6) led to significantly reduced plasma P4 concentrations. Likewise, Acosta et al. (2002) and Beatrice and Ulrich (2012) reported that the administration of PGF significantly decreased the volume of the CL and the blood P4 concentration of cyclic cows within 24 h of PGF administration.

Orally-treated rabbit does with Sun or Lin had the similar impact of PGF2α (0.5 mg dinoprostone) on increasing blood PG profile and decreasing blood P4 and this effect provided the opportunity to increased secretion of gonadotropin hormones from the pituitary and secretion estrogen from the ovary through inhibiting the effect of P4 on gonadotropin release. Furthermore, 17-β E2 level increased in the Sun and Lin tested groups as the same that found in the PGF2α group and this increase was significant in the Sun group than the PGF2α or Lin groups. Whereas, E2 hormone is necessary for influencing the follicular growth and production of mature ovum. Estradiol stimulates uterine secretion of PGF2α (Thatcher et al., 1995). Furthermore, E2 can increase the sensitivity of the CL to PGF2α, thus causing a more complete regression of the CL (Howard et al., 1990). From the study of Amira et al. (2011), rabbit does that fed on ration containing higher ratio of n6:n3 fatty acids with high arachidonic acid showed significantly (P<0.05) increased concentration of E2 and PGF2α in the plasma. Amira et al. (2011) showed that dietary PUFA supplementation can modify the plasma fatty acid profile and prostanooid synthesis. The significant of these modifications vary according to the type of polysaturated fat and the ratio of n-6:n3 fatty acid. On the other hand, PGF2α reduced the production of P4 because PUFA converted to PGF that caused an increase PGF concentration in the plasma with a reduction of the P4 synthesis. According to Smits (2010), the follicular phase begins after luteolysis and ends at ovulation. Gonadotropins, FSH and LH, released from the anterior pituitary, stimulate antral follicles to produce E2. The follicular phase is followed by E2 produced by developing follicles (Adams, 1999).

In addition, data of Sun revealed that the PGF2α, which produced in vivo from n6 (PG precursor) more effective on reproductive functions of rabbit does than that of the synthetic PGF2α. Also, the data revealed that Sun as a precursor of PGF2α, has a biological activity more than the Lin as a precursor of PGF2α. This finding was consistent with the finding of Lauderdale (2002) who found that in domestic animals, the most important and practical utility of PG appears to be PGF2α and the actions of PGF2α are mediated by the PGF receptor (Sugimoto et al., 1994).

Some reproductive performance

From Table 4, it could be concluded that treatment of aged rabbit does with eCG+PGF2α resulted in a significant increase in sexual receptivity by 8.1% above the group treated with eCG alone. On the other hand, treatment with Sun+eCG or Lin+eCG affected the libido, as the sexual receptivity increased significantly compared to the PGMs alone (by 23.8% for Sun and 19.9% for Lin, respectively) or PGMs+PGF2α (by 14.6% for Sun and 10.9% for Lin, respectively). Concerning the fertility rate, results demonstrated that rabbit does orally treated with Sun+eCG had the highest fertility rate (P<0.05) compared to the other experimental groups, which were not statistically different. This increase was 17.5 and 13.8% above the eCG and eCG+PGF2α groups, respectively. At the same time, treatment with eCG+PGF2α, Sun+eCG or Lin+eCG resulted in a significant increase (P<0.01) in litter size at birth than the does treated with eCG alone and these increases were 17.2, 43.7 and 21.9%, respectively. Likewise, treatment of rabbit does with Sun for 7 consecutive days prior to AI+PGF2α recorded the highest (P<0.01) bunny body weights at birth by approximately 22% than the control group. The present results indicated that Lin had a similar effect of PG injection on the studied traits, while Sun effect surpassed PG injection or Lin, which may be due to the fact that PGF2α – which is produced in vivo from Sun-n6 fatty acids (as a PG precursor) – is biologically more active than the synthetic PG and PGF2α – which are produced in vivo from Lin-n3 fatty acids. Increased biologic effect of PGF2α from Sun led to a decrease in blood P4 and an increase in blood 17-β E2 level, which is necessary for the growth and maturation of oocytes.

The results of the present study indicated that sexual receptivity was increased by using PGF2α injection or oral administration with Lin or Sun explaining the mating acceptance post-treatment. Dragan et al. (1996) reported that sexual receptivity of rabbit does injected with PG analogues is better than the control. Also, Hassanine (2000) found that the percentage of mating acceptance was 100% when does were injected by 2.5 mg of Lutalyse (PGF2α analogue) 48 h before natural mating with fertile bucks.

The results of fertility rate demonstrated that rabbits does treated with a synthetic PGF2α+eCG or with PG precursor (Sun or Lin)+PGMs had conception rate improved in comparison to the PGMs alone. The previous finding was in agreement with that of Facchin et al. (1992), who used PGF2α, 64 h before AI for oestrus synchronization of multiparous does. The PGF2α showed a positive effect on the performance compared to the eCG treated group. In a comparable trial, Abarino et al. (1995) observed that PGF2α improved the fertility rate in nulliparous and multipariparous inseminated does. Similarly, the results of Gogol (2009) showed that simultaneous treatment with PGF2α and eCG used for oestrus synchronization can increase reproductive performance in post-partum rabbit does, whereas, fertility rate and litter size were significantly increased in multiparous does treated with 20 U of eCG IM+0.5 PGF2α. According to Adamiak et al. (2005), PUFA content in follicular fluid is highly correlated to that of the diet and it is generally accepted that alterations in dietary fatty acid intake cause a similar shift in the fatty acid profile of the follicular fluid. A supplemental dietary lipid increases the size of the preovulatory follicle and its production of E2 (Zachut et al., 2008). The lipids stored within the oocyte and early embryo represents an important source of energy for the early embryo (McKeegan and Sturmey, 2012). Kowalska (2008) found that the female rabbits fed a complete diet fish oil supplemented had a quantitatively and qualitatively better milk fat content, higher fertility prolificacy values, and higher body weight of young rabbits at birth. The previous finding supports our results regarding the increase litter size, fertility and higher body weight at birth.

Conclusions

In conclusion, replacement of the injection of PGF2α to aged rabbit does by oral adminis-
tration with Sun improved reproductive hormone synthesis and secretion, reproductive performance, litter size and bunny body weight at birth. On the contrary, the results obtained by Lin did not differ significantly from PGF2α.

These treatments generally appear effective in older does at the end of their reproductive life or with reproductive problems.

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