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Does royal jelly have a reproductive effect on progesterone-treated goats?

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Abstract. *Introduction*: Some methods for inducing/synchronizing oestrus and ovulation in goats require the injection of equine chorionic gonadotrophin (eCG). The objective of this study was to determine whether royal jelly (RJ) can be a substitute for eCG in CIDR-treated dairy goats. *Methods*: Sixty-six primiparous goats received the CIDR device for twelve days in April. The eCG group (n=22) was injected with 200 IU of the gonadotropin when the intravaginal device was removed; the RJ group (n=22) received a daily intramuscular injection of 1 g of RJ during the period of CIDR treatment; the CIDR group (n=22) did not receive any additional treatment. A fourth group (Con, n=20) was used as control and received no treatment at all. Oestrus response, ovulation and fertility were recorded. *Results*: The oestrus percentage was higher in eCG (95%, $p<0.05$) than in the remaining groups (RJ, 41%; CIDR, 36% and Con, 0%) and occurred at shorter intervals (eCG, 35±2.3; RJ, 66±4.6; CIDR, 59±2; h±se; $p<0.05$). Ovulation was higher in eCG (91%, $p<0.05$), and RJ and CIDR were not different between them (50% vs 41%, $p>0.05$). No ovulation was recorded in the Con group. The percentage of fertile and birthing animals after 148-158 days was greater in the eCG (91%, $p<0.05$) compared to other groups (RJ, 36%; CIDR, 36%, Con, 0%), whereas it was not different between the RJ, CIDR and Con groups ($p>0.05$). *Conclusion*: There was no evidence of a reproductive effect of RJ on progesterone-treated goats.

Keywords: progesterone treatments; royal jelly; goats; oestrus induction.

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¿Tiene la jalea real un efecto reproductivo en cabras tratadas con progesterona?

Resumen. *Introducción*: Algunos métodos para la inducción/sincronización del estro/ovulación en cabras requieren de gonadotropina coriónica equina (eCG). El objetivo del estudio fue determinar si la jalea real (JR) puede ser un sustituto para la eCG en cabras lecheras tratadas con el dispositivo CIDR. *Métodos*: En el mes de abril, 66 cabras primerizas fueron tratadas con el dispositivo CIDR durante doce días. El grupo eCG (n=22) recibió una inyección de 200 UI de eCG al retiro del dispositivo. El grupo JR (n=22) recibió una inyección intramuscular diaria de 1 g de JR durante el periodo de uso del CIDR. El grupo CIDR (n=22) no recibió tratamiento adicional. Se incluyó un grupo control (Con, n=20) que no recibió tratamiento alguno durante todo el estudio. Se registró la respuesta estral, ovulación y la fertilidad. *Resultados*: El porcentaje de animales en estro fue mayor en el grupo eCG (95%, $p<0.05$) que en los otros (RJ, 41%; CIDR, 36% and Con, 0%) y se presentó en un intervalo menor (eCG, 35±2.3; RJ, 66±4.6; CIDR, 59±2; h±ee; $p<0.05$). El porcentaje de ovulación fue mayor en el grupo eCG (91%, $p<0.05$); no se encontraron diferencias entre JR y CIDR (50% vs 41%, $p>0.05$). No se registró ovulación en el grupo Con. El porcentaje de fertilidad fue mayor en el grupo eCG (91%, $p<0.05$) comparado con los otros grupos (JR, 36%; CIDR, 36%, Con, 0%), mientras que JR y CIDR no fueron diferentes ($p>0.05$). *Conclusión*: No se encontró evidencia de un efecto reproductivo de la JR en cabras tratadas con progesterona.

Palabras clave: progesterona, cabras, jalea real, inducción estral.

A geleia real tem efeito reprodutivo em cabras tratadas com progesterona?

Resumo. *Introdução*: alguns métodos para induzir/sincronizar o estro/ovulação em cabras requerem de gonadotropina coriônica equina (eCG). O objetivo deste estudo é determinar se a geleia real (GR) pode ser uma substituta para a eCG em cabras leiteiras tratadas com o dispositivo CIDR. *Métodos*: em abril, 66 cabras principiantes foram tratadas com o dispositivo CIDR durante 12 dias. O grupo eCG (n=22) recebeu uma injeção de 200 UI de eCG na retirada do dispositivo. O grupo GR (n=22) recebeu uma injeção intramuscular diária de 1 g de GR durante o período de uso do CIDR. O grupo CIDR (n=22) não recebeu tratamento adicional. Um grupo controle (Con, n=20) foi incluído, o qual não recebeu tratamento durante todo o estudo. Foi registrada a resposta estral, ovulação e fertilidade. *Resultados*: a porcentagem de animais em estro foi maior no grupo eCG (95%, $p<0,05$) do que nos outros (GR, 41%; CIDR, 36% e Con, 0%) e foi apresentada em um intervalo menor (eCG, 35±2,3; GR, 66±4,6; CIDR, 59±2; h±ee; $p<0,05$). A porcentagem de ovulação foi maior no grupo eCG (91%, $p<0,05$); não foram encontradas diferenças entre GR e CIDR (50% vs 41%, $p>0,05$). Não foi registrada ovulação no grupo Con. A porcentagem de fertilidade foi maior no grupo eCG (91%, $p<0,05$) em relação aos outros grupos (GR, 36%; CIDR, 36%, Con, 0%), enquanto GR e CIDR não foram diferentes ($p>0,05$). *Conclusões*: não foi verificada evidência de um efeito reprodutivo da GR em cabras tratadas com progesterona.

Palavras-chave: progesterona, cabras, geleia real, indução estral.

Introduction

The seasonal reproductive pattern of goats generates irregular availability in annual production, leading to a low availability of milk and its by-products during periods when the market demand is strong and higher prices would be paid [1]. This has led to the development of techniques to stimulate reproduction during periods in which breeding would not naturally occur [2]. The use of intravaginal progesterone devices combined with gonadotropins (equine chorionic gonadotropin; eCG) is one of the most common and successful methods for inducing/synchronizing oestrus and ovulation throughout the year [3-6]. The CIDR device (controlled internal drug release, CIDR®) contains progesterone and it is used for periods of 9-14 days with satisfactory results [3,5,7].

During the anoestrous season, an injection of eCG is necessary to promote a greater follicle development, oestral response and ovulation rate after progesterone-progestogen treatments [2,4,5]. During this time of the year, progestogen-treated sheep may not respond without eCG [8-11]. In dairy goats [4,6] and sheep [5,10] doses of up to 1000 IU of eCG are used with progesterone treatments [12]. Even when synchronizing oestrous activity, better results are obtained when eCG is used than in controls [13-15]. In spite of its effectiveness, this treatment is expensive for traditional farming. Moreover, this gonadotropin induces an immune response that affects its future effectiveness [2,16-18].

Royal jelly (RJ) is a substance produced from the pharyngeal glands of the bee (*Apis mellifera*). It contains high concentrations of proteins, amino acids, lipids, vitamins and sugars (19-21] and it has been shown to possess nutritional, pharmacological and reproductive properties in different species [20,22-24]. In humans, it has been proposed that RJ reduces menopausal symptoms through its apparent oestrogenic role [25], and others have suggested that it contains several other hormonal-type activities [26]. In heat stressed male rats [27] and rabbits [28] RJ improves testosterone levels and sperm motility and concentration. Moreover, RJ promotes folliculogenesis and increases ovarian hormones in immature rats [29].

Used *in vitro*, RJ clearly shows an oestrogenic function [30] and increases goat [31] and sheep [32,33] oocyte maturation. In sheep, RJ has also been used as an eCG substitute in progesterone-based treatments. In several studies, RJ provoked oestrus and ovulatory responses when injected or administered orally during the progesterone treatment [34-36]. These studies used doses of 250 mg/day and suggested that higher doses could improve the oestral response [34]. However, the results obtained for goats were less clear [37], as an unexpected high response in control animals resulted in an ambiguous conclusion. The aim of the present study was to determine whether RJ might be a substitute for eCG in progesterone-treated dairy goats during their non-breeding season. It was hypothesised that an i.m. injection of 1 g/day of RJ would induce results comparable to those obtained using eCG and higher than using nothing in progesterone-treated goats.

Materials and methods

Location and animals

The study was conducted at an experimental farm located 150 km north of Mexico City. The experimental protocol was approved by the Internal Animal Ethics Committee of the Faculty of Veterinary Medicine of the National Autonomous University of Mexico. A total of 86 primiparous dairy goats were included in the study (French Alpine, Toggenburg and Saanen). Animals were handled according to farm routine and fed three times daily with alfalfa hay and a commercial concentrate. Clean water was always available.

Treatments

During April, 66 primiparous goats were treated during 12 days with the CIDR device and randomly assigned to one of three treatments. The eCG group (n=22) was i.m. injected with 200 IU of eCG when the device was removed. The RJ group (n=22) was i.m. injected daily with RJ (1 g) throughout the 12 days of CIDR use, starting at the time of insertion [35-37]. The CIDR group (n=22) received no additional treatment. A fourth group was included as a control (Con, n=20) and received no treatment at all. The day of CIDR removal was considered as time 0. Animals were kept in different adjacent pens at least 150 m^2 from each other.

RJ was obtained directly from the producer and used within two months of its harvesting from the hives. It was diluted using bi-distilled water (2 ml water/1 g of RJ) before starting the study and was kept refrigerated until use. Before dilution, RJ was filtered using milk filters (DeLaval® México) to eliminate impurities. It was injected in different places to avoid the possibility of minor local reactions [37]: in semitendinosus, semimembranosus muscle and on both rear leg biceps.

Ovulation

Blood samples were taken from twelve goats per group with the aim of determining progesterone as evidence of ovulation before and after treatments. A weekly sample was taken during three weeks before initiating treatments. Three days after the CIDR withdrawal, a daily six-day sampling period started. All samples were immediately centrifuged, and serum separated and frozen until analyzed by a solid phase radioimmunoassay (Coat-A-Count, Siemens®). Assay sensitivity was 0.02 ng/ml and the intra-assay coefficient of variation was 3.3%. The ovulation was considered to have occurred when progesterone values were above 1 ng/ml in three consecutive samples.

Oestrus behaviour

Fifteen hours after removing CIDRs, oestrus behaviour was recorded every 6 h during 4 days with the aid of an aproned male introduced for about 5 min per pen each time. Goats in oestrus were mated with the selected male at least twice, 12 hours apart. Subsequently, for ten days after the CIDR removal, oestrus behaviour was measured once per day.

Pregnancies

Pregnancy was diagnosed 45 days after CIDR removal using abdominal ultrasound. Births occurring 148-158 days after device removal were registered to confirm the results. It was considered that birthing during the period resulted from a fertile ovulation as a positive response to treatments.

Analysis

The following parameters were measured and compared: percentage of does in oestrus, interval to oestrus, percentage of does ovulating, percentage of pregnancy. ANOVA, Tukey's tests and Fisher's exact test were used (PROC GLM and PROC FREQ) in SAS v9.

Results

Values of progesterone before treatments confirmed the anoestrous condition. All animals had progesterone levels <1ng/mL.

Results are shown in Table 1. The eCG group had the highest percentage of oestrus, ovulation and pregnancies ($p<0.05$). None of the measurements were different between RJ and CIDR groups ($p>0.05$). The interval to oestrus was longer ($p<0.05$) in the RJ and CIDR group than in the eCG group (Table 1).

4. Discussion

According to this study, no significant benefit was observed from daily i.m. administration of 1 g of RJ

Table 1. Reproductive response of goats after treatment with the progesterone CIDR device for 12 days and an injection of 200 IU of equine chorionic gonadotrophin (eCG) or daily doses of 1 g of royal jelly (RJ). Control group received no treatment at all.

	eCG n=22	RJ n=22	CIDR n=22	Control n=20
Goats in oestrus (%)	95.5[a]	41[b]	36.4[b]	0[c]
Interval to oestrus (h)	35.1±2.3[a]	66.5±4.6[b]	59.3±2[b]	--
Goats ovulating (n=12; %)	91.6[a]	50[b]	41.6[b]	0[c]
Goats kidding (%)	91[a]	36.4[b]	36.4[b]	0[c]
Kids/parturition	2.5±0.4[a]	2.1±0.3[a]	1.8±0.3[a]	--

[a,b,c] Values with different superscripts within a row are significantly different ($p<0.05$).

Source: own work

in terms of oestrus, ovulation and fertility in primiparous goats during their non-breeding season. These results are inconsistent with those of Husein and Kridli [34] and Kridli et al. [35] in sheep, where at least 80% of ewes that received 250 mg/day of RJ during their progesterone treatment went into oestrus faster than when only progesterone was administered [34].

According to Husein and Kridli [34], 250 mg of RJ may have enhanced follicular development, thus increasing oestradiol secretion. The authors suggested that high doses of RJ would help increase oestrus manifestation by enhancing follicular development. The study presented therein injected 1 g/day and was unable to induce ovulation and oestrus in similar proportions to treatment with eCG or higher than CIDR use only. Furthermore, published papers on sheep do not clarify the oestrus response of control animals [36], which could be associated either with being in permanent contact with the male after progesterone treatment or to annual variations in the anoestrous depth, which would allow reproductive activity to occur after the use of intravaginal devices alone [38-40]. The presence of cycling animals at the beginning of the experiment [36] should also be considered to potentially have a confusing impact on the results. In our experiment, no goat was cycling before the study.

On the other hand, our results resemble those of Kridli and Al-Khetib [41] in sheep. Those authors concluded that eCG but not RJ had an effect in improving oestrus expression. In other cases, using RJ was not associated with any reproductive stimulatory effect in rabbits [42].

The oestrus interval in goats from the RJ and CIDR group was almost doubled compared to that in the eCG group. This is inconsistent with reports by Husein and Hadad [36], where eCG and RJ-treated (400 mg) sheep entered into oestrus at similar intervals after progesterone treatment withdrawal. Husein and Kridli [34] also found a lower oestrus interval when using i.m. or oral RJ in sheep. On the contrary, Kridli et al. [35] found no difference in oestrus intervals between RJ and control ewes, similar to our results. In our study, the oestrus interval was not different between RJ and CIDR treatments, making it difficult to suggest a stimulating effect of RJ at the follicular level. Similar to rich diets [43,44], it has been proposed that RJ induces follicular development due to its nutritional content [36]. The stimulated follicular development would then induce a higher production of oestradiol, inducing oestrus behaviour [45]. However, Kridli and Al-Khetib [41] found contradictory results. In their study [41], increasing doses of RJ (250, 500 and 750 mg/day) in progesterone-treated sheep did not lead to a higher response compared to control animals. Furthermore, control animals treated only with a CIDR device, responded with 100% of oestrus behaviour [41]; like another experience with RJ on young goats during their non-breeding season [37]. Thus, to our knowledge, the positive results described in sheep have not been confirmed or expanded to goats.

RJ is considered to have nutritional qualities that contribute to the organism's nutritional balance [46]. RJ is rich in vitamins, minerals, proteins and amino acids [21,47,48], and this helps to explain some of its effects as a cellular antioxidant [49-51] and in increasing sperm activity [52]. The role of RJ in alleviating certain menopausal syndromes [53] suggests an oestrogenic effect, and *in vivo* and *in vitro* experiments confirm that RJ joins oestradiol receptors and induces transcription like steroids [25,30], actions that are blocked by an oestradiol antagonist [25,54]. It is clear more research is needed to clarify a possible practical use for RJ in animal reproduction.

Progesterone-based treatments to induce oestrus and ovulation in goats require a gonadotropic stimulus. When a gonadotropic stimulus is not provided, results tend to be low or absent [4,40,55]. New methods to evoke such a stimulus are needed. In progesterone-treated goats, the male effect has been shown to be an efficient method to substitute eCG and to induce an oestrus response, like that obtained with eCG [56]; this significantly reduces treatment costs as well. In the present study measuring oestrus, ovulation and fertility responses, we were unable to find evidence to suggest a gonadotropic effect of RJ in goats treated with the CIDR device.

Conclusion

We conclude that an i.m. injection of 1 g/day of RJ during the CIDR treatment did not enhance reproductive fitness compared to eCG, as evaluated by oestrous behaviour, ovulation and pregnancy rates in primiparous goats during their anoestrous season.

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