Research Article

BIOMEDICAL Journal of Scientific & Technical Research

ISSN: 2574 -1241

DOI: 10.26717/BJSTR.2023.53.008391

Progesterone Present in Royal Jelly can Successfully Synchronize Oestrus in Goats

Castro Chávez N[1], Chávez Güitrón LE[2], Ratti Vázquez LD[1], Guerrero Netro HM[1], Gutiérrez Molotla J[1] and Balcázar Sánchez JA[1]*

[1]*Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Avenida Universidad 3000, México*

[2]*División Químico-Biológicas, Universidad Tecnológica de Tecámac Carretera Federal MéxicoPachuca km 37.5, Predio Sierra Hermosa, México*

***Corresponding author:** Balcázar Sánchez JA, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Avenida Universidad 3000, Coyoacán, México, Ciudad de México, 04510, México

ARTICLE INFO

Received: October 10, 2023

Published: October 18, 2023

Citation: Castro Chávez N, Chávez Güitrón LE, Ratti Vázquez LD, Guerrero Netro HM, Gutiérrez Molotla J and Balcázar Sánchez JA. Progesterone Present in Royal Jelly can Successfully Synchronize Oestrus in Goats. Biomed J Sci & Tech Res 53(3)-2023. BJSTR. MS.ID.008391.

ABSTRACT

Royal Jelly (RJ) is a natural beehive product that serves as food for the queen bee through the larval period. RJ is known for having estrogenic activity and has a unique composition. However, the components that mediate RJ´s estrogenic effect remain unknown. The present study evaluated if RJ has steroidogenic hormones that are biologically relevant when applied intravaginally for oestrus synchronization in goats.

In the first trial, goats were ovariectomized and treated for six days with either a CIDR device or a RJ-impregnated intravaginal sponge. In the second trial, goats were treated for 6 days with a CIDR device or RJ-impregnated intravaginal sponges. On day 5 of treatment, each goat received an injection of PGF2α and eCG. Blood samples were collected daily in both trials. RJ and serum concentrations of oestradiol, progesterone, and testosterone were measured by ELISA. Oestrus presentation was evaluated, and pregnancy rates were determined by ultrasonography. RJ contains progesterone, testosterone, and oestradiol. When RJ was applied intravaginally in ovariectomized goats, progesterone levels in serum significantly increased. Additionally, during oestrus synchronization, RJ had no significant differences in progesterone, oestradiol, and testosterone serum levels when compared to CIDR treatment. Furthermore, RJ significantly increased the number of matings and reduced the average time for detection of standing oestrus. We conclude that RJ contains steroidogenic hormones that can synchronize oestrus when applied intravaginally.

Keywords: Royal Jelly; CIDR; Goat; Synchronization; Steroid Hormones; Ovariectomy

Introduction

Royal Jelly is a natural beehive product synthesized in the hypopharyngeal and mandibular glands of young nurse worker bees (Apis mellifera) which serves as food for the queen bee through the larval period. RJ comprises 60- 70% water, 10-12% carbohydrates, 12-15% proteins, and 3-7% lipids [1]. Based on its unique composition, RJ is attracting a progressive growing interest due to its health-beneficial properties such as antioxidant, antimicrobial, neuroprotective, anti-inflammatory, immunomodulatory, antiaging, and estrogenic activities [2]. RJ has shown its estrogenic effects both in vitro and in vivo. In humans, RJ reduces premenstrual syndrome, and it is used by postmenopausal women for the treatment of menopause-related complications [3,4]. In vitro, supplementation of maturation media with RJ significantly increased cumulus cell expansion and oocyte maturation rates in cows, goats, and sheep [5-7]. In goat and sheep oocytes, RJ significantly reduces mRNA expression of apoptosis-related genes such as p53 and Bax and increases the transcript abundance of the anti-apoptotic gene Bcl-2 [5,6]. Additionally, glutathione content augmented linearly with increasing concentrations of RJ, which is linked to the developmental potencial of the oocyte and the future embryo [5,8]. RJ significantly improved the embryo development of RJ-treated oocytes in different species. In sheep parthenogenic embryos, RJ significantly increased blastocyst rates [6]. In goats, RJ improves the per-

centage of cleaved embryos and blastocyst rates at concentrations of 5 and 10 mg/ml [5]. Furthermore, RJ has been widely used as a method to improve in vivo conception rates in sheep [9-11] and rats [12]. RJ estrogenic activity has been related to its affinity to bind to both estradiol receptors but, this affinity is low when compared to other chemicals, which might suggest the presence of other RJ components related with estrogenic activity [13]. Thererfore, we hypothesize that RJ contains steroidogenic hormones that could be functionally relevant during the oestrus synchronization in goats.

Material and Methods

Study Area

All experiments were performed in ¨Centro de Enseñanza Práctica e Investigación en Producción y Salud Animal¨ (CEPIPSA) located in Mexico City, at 19°12'33.4"N 99°09'10.0"W and an average elevation of 2,760 masl.

Royal Jelly-Impregnated Intravaginal Sponges

Fresh royal jelly was obtained from the local beekeeper (El Fenix, in San Bartolo, Mexico City) just before experiments started (early November). Polyurethane sponges (3 cm diameter x 3.8 cm high) were impregnated with 10g of RJ and then dehydrated by placing them in a dry culture oven (Brand: BG Laboratories and model E33) at 30°C for 48 hours.

Hormonal Analyses

Royal jelly and serum concentrations levels of estradiol, progesterone, and testosterone were measured using an ELISA Kit (Progesterone DRG Kit, Testosterone DRG Kit, and Estradiol DRG Kit). All measurements were taken according to the manufacturer's instructions. The detection limit was 0.045ng/mL for progesterone, 9.714 pg/mL for estradiol, and 0.083 ng/mL for testosterone.

Blood samples were collected daily via jugular puncture. Sample collection started two days before treatment and finished one day after CIDR or intravaginal sponge withdrawal. Whole blood tubes were centrifuged at 3000 rpm for 10 minutes to separate the serum. The serum samples were stored at -18°C until use.

Estrus´s presentation was evaluated using two bucks. Monitoring for estrus started at CIDR or intravaginal sponge removal and was performed twice a day. Estrus duration (h) was measured from male acceptation until the females rejected the mating. The pregnancy in these animals was determined by ultrasonography 40 days after the natural mating.

Statistical Analysis

The variables that were evaluated and compared among groups were progesterone, testosterone and estradiol serum concentrations, , average time for the manifestation of post-treatment estrus, duration of estrus, number of services, prolificacy and percentage: of goats in estrus, of return to estrus, conception and fertility.

An analysis of variance was carried out for a completely randomized model and a Tukey test for the analysis of the following variables: serum progesterone concentrations, serum testosterone concentrations and serum estradiol concentrations.

The variables of matings, manifestation of post-treatment estrus, duration of estrus were analyzed with a test for a completely randomized model for adjusted means, ANDEVA. While for the analysis of estrus presentation, conception rate, an exact Fisher test was performed. All statistics analysis were performed by STATISTICA 7.0 for Windows software.

Results

To determine whether royal jelly contains steroidogenic hormones, we first measure the levels of progesterone, oestradiol, and testosterone. Summary data are presented in (Table 1). We then assessed if steroidogenic hormones from RJ could be absorbed from the vagina and transferred to the bloodstream. Ovariectomized goats were used to compare the absorption of progesterone present in RJ versus a CIDR device. Goats with RJ-impregnated intravaginal sponges had a gradual increase in progesterone levels found in plasma. In contrast, the CIDR device resulted in a rapid increase in progesterone concentrations (Figure 1). The functional relevance of the steroidogenic hormones present in RJ was assessed during oestrus synchronization in goats. Adult goats were synchronized using a CIDR device or a sponge impregnated with RJ. Animals treated with RJ or CIDR device had no significant differences in the concentration of progesterone, oestradiol, and testosterone ($P>0.05$) (Figure 2). Regarding the oestrus onset, goats treated with RJ showed the earliest average time for oestrus manifestation and a significantly higher number of matings when compared to goats treated with the CIDR device ($P<0.01$). In both groups, we found no difference in the number of animals in heat or the duration of standing oestrus ($P>0.05$). However, there was a tendency for higher conception rates in goats treated with RJ when compared to animals treated with a CIDR device (Table 2).

Table 1: The concentration of steroidogenic hormones presents in fresh royal jelly.

Hormone	Concentration
Oestradiol	5.4 ng/mL
Testosterone	6.76 ng/mL
Progesterone	17.79 ng/mL

 DOI: 10.26717/BJSTR.2023.53.008391

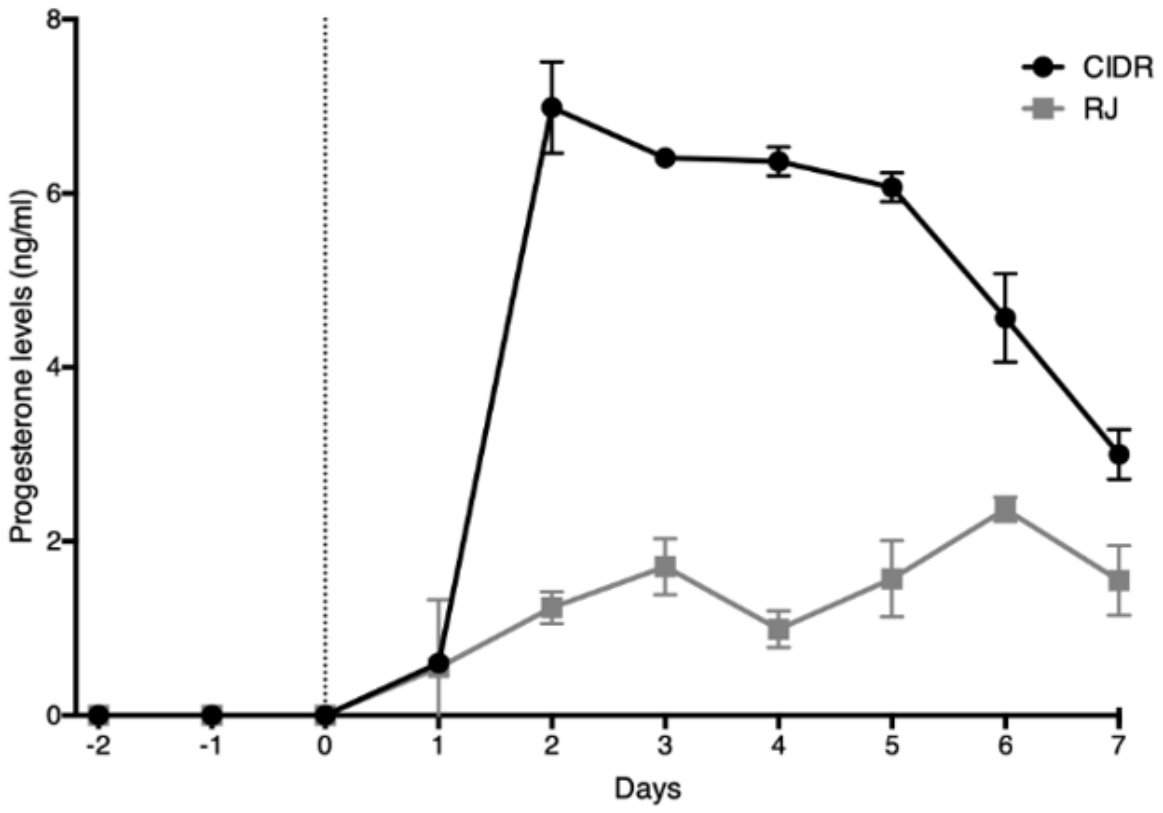

Figure 1: Average concentration of progesterone (ng/m) in blood serum from ovariectomized goats. Two months after ovariectomy, goats were treated with either a CIDR device or a royal jelly impregnated sponge. Blood samples were taken daily starting two days before treatment and finishing one day after CIDR / royal jelly impregnated sponge removal. Progesterone levels were determined by ELISA.

Table 2.

Variable	CIDR	RJ
Average time for detection of standing oestrus after CIDR/ sponge removal	41.5±1.54 h^{a*}	25.6±1.10 h^{b*}
Goats in oestrus (%)	100% (9/9)^{a}	100% (7/7)^{a}
Duration of the standing oestrus	12.27±0.13 h^{a}	12.28±0.10 h^{a}
Matings	1.10±0.15^{a+}	2.56±0.18^{b+}
Conception rate (%)	66.67% (6/9)^{a}	85.71% (6/7)^{a}

Note: Different letters indicate significant differences *(P<0.05) + (P<0.01)

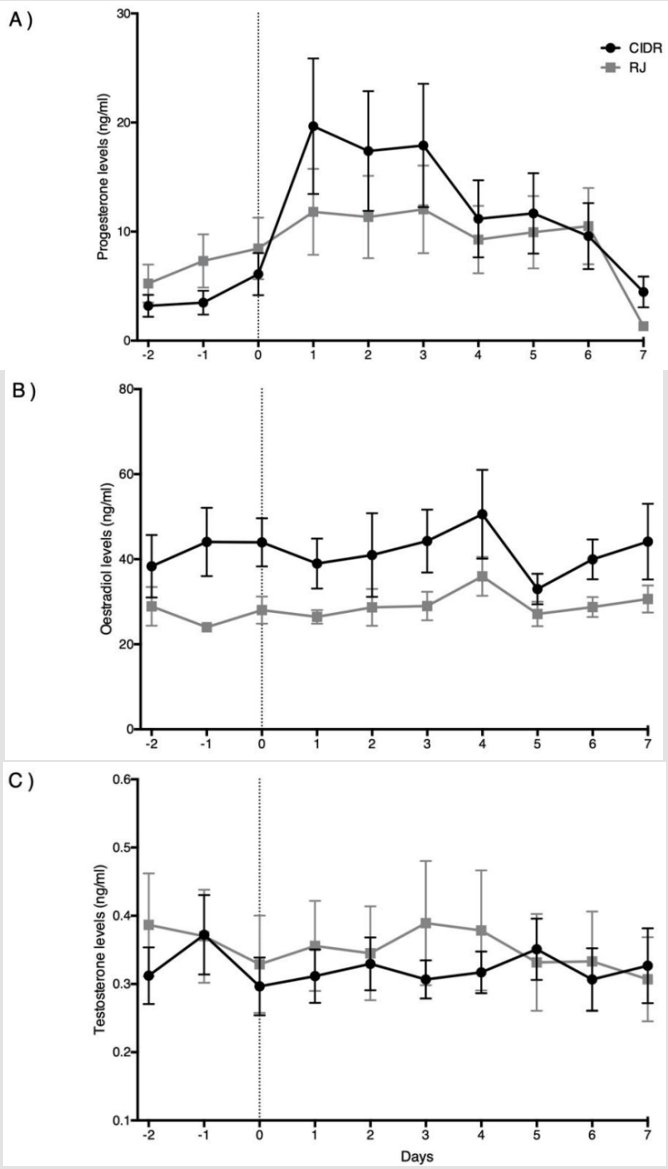

Figure 2: The average concentration of steroidogenic hormones (ng/mL) in adult goats 147 synchronized with a CIDR device or an intravaginal sponge impregnated with RJ. Adult 148 goats were synchronized with a CIDR device or an intravaginal sponge impregnated 149 with royal jelly for 6 days. Blood samples were taken daily starting two days before 150 treatment and finishing one day after CIDR / RJ-impregnated sponge removal. 151 Progesterone
(A) Oestradiol
(B) Testostorene
(C) Leves were determined by ELISA.

Discussion

RJ has estrogenic activity in different species but, the components of RJ that mediate this activity remain unknown. In this study, we show that RJ contains steroidogenic hormones (progesterone, testosterone, and oestradiol) that when applied intravaginally, can augment the levels of sterodoigenic hormones in the blood, reducing the average time for detection of standing oestrus and synchronizing the oestrus in the goat. RJ estrogenic activity has been related to its affinity to bind to both estradiol receptors (ER), ERα, and ERβ. In rats, RJ can inhibit the binding of E2 with ERs in a dose-dependent manner [14]. A possible explanation for this ER affinity is the fatty acids present in RJ (10-hydroxy-2-decanoic, 3,10-dihydroxydecanoic, and sebacic acid) that can modulate the recruitment of ERα, ERβ and are co-activators to target genes [13]. However, RJ affinity to the ER is low when

compared to other chemicals like diethylstilbestrol and phytoestrogens, which might suggest the presence of other RJ components related with estrogenic activity [14]. Androgens act as precursors for all estrogens. In humans, six-months ingestion of RJ can significantly increase the levels of testosterone in healthy patients [15].

Vittek and Slomiany first reported the presence of testosterone in RJ, they tested native RJ from different years and found the presence of testosterone in an average concentration of 11.63 ng/ml [16]. According to our data, the RJ used in our experiments had around 6.76 ng/mL of testosterone, half of the concentration previously reported by Vitek and Slomiany. These results were as expected, previous studies report that RJ composition varies significantly (for both fresh and dehydrated samples) due to floral periods, harvest time and storage conditions [17-20]. Unfortunately, the presence of other steroid hormones in RJ had not been assessed. Nevertheless, previous research has shown that RJ can significantly increase the levels of progesterone in rats and ewes [11,21]. When applied vaginally, RJ cream is more effective than estrogens in improving sexual and urinary function in postmenopausal women, suggesting a possible absorption of RJ components by the vaginal wall [22]. We observed in ovariectomized goats treated with intravaginal RJ an increase in circulating progesterone levels (2ng/ml) that were higher than those reported by Rubianes et al. as sub-luteal levels (0.4±0.1 ng/ml)[23]. Incidentally, earlier studies stated that to synchronize oestrus, progesterone concentration should be above sub-luteal levels for 3 or 4 days [23]. Thus, the data suggest that RJ intravaginally could be used to synchronize oestrus in goats. In the present study, we calculated the concentration of progesterone contained in each RJimpregnated sponge (177 ng).

The CIDR device contains 300mg of progesterone yet, earlier investigations reported that the same CIDR device can be re-used up to three times with no significant changes in oestrus duration, the interval from device removal to the onset of oestrus, fertility, and prolificacy after artificial insemination [24]. These results might indicate that lower doses of progesterone than those present in CIDR devices are enough to synchronize the oestrus in goats [24]. Interestingly, our RJ-treated group had a reduction in the average time for detection of standing oestrus after sponge removal when compared to the CIDR-treated group. Prior research reported this reduction in the interval to the onset of oestrus in sheep treated with intramuscular or oral administration of RJ for 12 days [11]. A possible explanation is that RJ can significantly increase follicular development in sheep that results in higher levels of estrogens and conception rates [11]. In our results, the conception rates were not significantly different, but the animals had a tendency of presenting double pregnancies and had significanly more matings. The use of intravaginal sponges can cause alterations in the vaginal microbiota and as a result, decrease the sexual attractiveness as previously reported in ewes [25]. However, RJ is known for its bactericidal effects that might reduce the overgrowth of bacteria found in the vagina and therefore increase the number of matings [26].

Conclusion

RJ contains steroidogenic hormones such as progesterone that can have a biological function when applied intravaginally during oestrus synchronization in goats. Additionally, RJ can reduce the average time for detecting standing oestrus and increase the number of matings when compared to CIDR. Nevertheless, further research is needed to understand the mechanisms of action of RJ in the ovary.

Acknowledgements

The authors would like to thank MVZ Yazmin Ivonne Arriaga Avilés and M. en C. Ana Delia Rodríguez Cortéz, for their valuable help in the experiment.

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ISSN: 2574-1241

DOI: 10.26717/BJSTR.2023.53.008391

Balcázar Sánchez JA. Biomed J Sci & Tech Res