# Effect of local Iraqi goat semen supplemented with Alcoholic hemp extract on pregnancy and fecundity rate outside the breeding season

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## Abstract

Prolonged semen productivity during chilled storage is a vital step in the goat artificial insemination (AI) technique, predominantly during the non-breeding season. The objective of this study was to investigate the effect of adding hemp alcoholic extract to the Tris-egg yolk diluent on the fecundity and pregnancy rate of local Iraqi goat semen. Three local male and 11 multiparous female goats were used in this study. Semen samples were collected, diluted from all males, and then divided into three groups: T0= control, T1= 1% 100µL, T2=1% 200µL, hemp alcoholic extract. All semen samples were kept in a refrigerator (5°C) until insemination. The estrous cycle was synchronized using a vaginal sponge containing 40 mg of Flurogestone Acetate (FGA) for 12 days. On the day of sponge removal, all animals were intramuscularly injected with 125 µg prostaglandin F2α and 400 IU equine chorionic gonadotropin (eCG). 44 hours following sponge removal, a single vaginal insemination was performed by using fixed time AI (FTAI). Transabdominal ultrasonography was used for pregnancy diagnosis for all animals at 47 days following insemination. In addition, the fecundity rate was estimated following parturition. The results of the current study showed a pregnancy rate of 25% in T2, compared to 0% in both T0 and T1. In addition, the fecundity rate was significantly higher in T2 compared to other groups ($P < 0.05$). In conclusion, local goat semen supplemented with a specific concentration of hemp alcoholic extract yielded promising fertility in local Iraqi goats during the non-breeding season. Additional research is warranted to evaluate the protective antioxidant role of hemp through a large sample size.

**Keywords:** hemp, Local Goat, Fixed-time artificial insemination, non-breeding season, fertility

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## Introduction

Artificial Insemination (AI) remains the most effective tool for genetic improvement. Yet, chilling semen induces oxidative stress, leading to lipid peroxidation of the sperm's plasma membrane [1]. In developing countries, goats are an essential factor of livestock and are important for food security. The benefits of goats come from their small size, low maintenance, and ability to live in harsh climates [2]. However, like sheep, goats exhibit polyestrous seasonality, meaning they do not ovulate during long daylight hours, which limits their productivity throughout the year [3,4]. In Iraq, both the non-breeding season and heat stress negatively affect female semen viability and fertility, thereby reducing farm profitability [5]. In goats, AI remains an effective assisted reproductive technique for the widespread dissemination of superior genetic components [6]. However, the chilling semen process induces oxidative stress, leading to lipid peroxidation due to the plasma membrane's high content of polyunsaturated fatty acids (PUFAs) [7,8 .[

Hemp, Cannabis sativa cultivars, is a family member of cannabis [9]. It has been well-documented to possess antioxidant properties that scavenge free radicals and reduce the risk of sperm damage following ejaculation, due to its rich phytocannabinoid and polyphenol content [1]. Different administrations of hemp have shown different outcomes in different species. Alagbonsi and his coworker (2019) found that male Wistar rats treated orally with hemp (2 -10 mg/kg) resulted in increased semen and antioxidant parameters [10]. In addition, Meador et al. (2024) found that adding SHB (the fibrous residue of the hemp plant, also known as hemp biomass) to the feed at up to 20% for rams for up to 8 weeks did not adversely affect the tested fertility parameters [11]. In addition, in equines, hemp supplementation resulted in increased mating behavior, libido, and spermatogenesis parameters [12–14]. However, in humans, chronic supplementation of hemp in vitro negatively affects sperm viability [15]. According to the previous research, hemp might improve the sperm parameters, which

may be attributed to its potent antioxidant properties, which play a

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key role in this process [16]. To our knowledge, it is unknown whether the direct addition of hemp extract as a cryoprotective agent to goat semen has been studied. This study aims to evaluate, for the first time, the efficacy of an alcoholic hemp extract in improving the fertility outcomes of chilled local Iraqi goat semen during the anestrus season.

## Materials and Methods

### Animals

The current study was conducted in one of the local breeders' fields in Babylon, Iraq. Ethical approval was provided by Al-Qasim Green University's ethics committee (Agreement #4897, 2024). Three local male (1-3 years, 30-40 kg) and 11 multiparous does (2-3 years, 25-35 kg) goats were used in this study.

### Semen collection and preparation

Semen was collected from three male local goats aged 1–3 years and weighing 30–45 kg using an artificial vagina designed by the researcher. The experiment was conducted from 10-7-2025 to 24-12-2025. After collection, the semen was pooled to eliminate intermale differences. A (1–10) ml dilution of a Tris-yolk expander was used, consisting of (3.63 g Tris-yolk, 1.99 g citric acid, 1 g fructose, 10 ml egg yolk, 100 mg antibiotic, and 100 ml distilled water). Following dilution, samples were divided into three groups as follows: T0 = control group (without additives), T1 = T2= 1% 100µL, T3=1% 200µL hemp alcoholic extract. Semen treatment selection was prepared based on unpublished previous data. All samples were stored in the refrigerator at 5°C until insemination on the same day of collection.

### Estrus Synchronization in Goats

Regarding estrus synchronization, 11 female goats were selected that had previously given birth to ensure the possibility of pregnancy and to verify their health, free from diseases such as lameness, coughing, shortness of breath, nasal discharge, fever, and mastitis. After the animals arrived at the pen, a pregnancy test was performed using an ultrasound. The does were randomly divided into three groups as follows: a control group (n=3 animals), group T1 (n=4), and group

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T2 (n=4). A vaginal sponge containing 40 mg of Flugestone Acetate (China) was used for 12 days. Two days prior to sponge removal, all animals were injected intramuscularly with 125µg prostaglandin F2α (PROSTAGENIX, Chiena) for the remaining corpus luteum removal. At the time of sponge removal, 400 IU/ doe of equine chorionic gonadotropin (eCG) was injected for all animals.

## Fixed-time artificial insemination (FTAI)

Fixed time artificial insemination (FTAI) was performed 46 hours following sponge removal [17]. Female goats were inseminated in the vagina with 1 ml of diluted semen. The artificial insemination procedure was then performed using an artificial insemination device (Medsinglong Global Co., Ltd., China). Briefly, the female's body was lifted from behind and securely held. The device tube, equipped with a camera and light source to visualize the apical openings of the cervical canal and the semen deposition site, was inserted into the vagina. This site contained a thin tube into which the semen was introduced and then pushed using a special pump (20 ml syringe). After the semen was dropped, the device was slowly withdrawn and thoroughly cleaned for reuse by all the experimental animals. A pregnancy diagnosis was performed on day 47 post artificial insemination using an ultrasound equipped with a convex prob 3.5 MHz linear array transducer. The probe was placed on the abdominal area, and the goats were classified as pregnant when fetuses were visualized (Shisun Medical Technology Co., Ltd., China) to determine the pregnancy rate. Following lambing, the fecundity rate (number of lambs borne per goat) was determined according to the following equation [18].

Fecundity % = number of births / Total number of does ×100.

## Statistical analysis

One-way analysis of variance (ANOVA) was used to analyze the parameters, e.g., pregnancy and fecundity rate among treatments, using the SAS program [19]. Chi-squared test was used to compare pregnancy and fecundity rates across treatments. The significant difference was ($P < 0.05$).

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## Results and discussion

In the current study, statistical analysis showed that the pregnancy rate was significantly higher ($P < 0.05$) in T2 (25%) than in T1 (0%) and T0 (0%) during the non-breeding season (Figure 1).

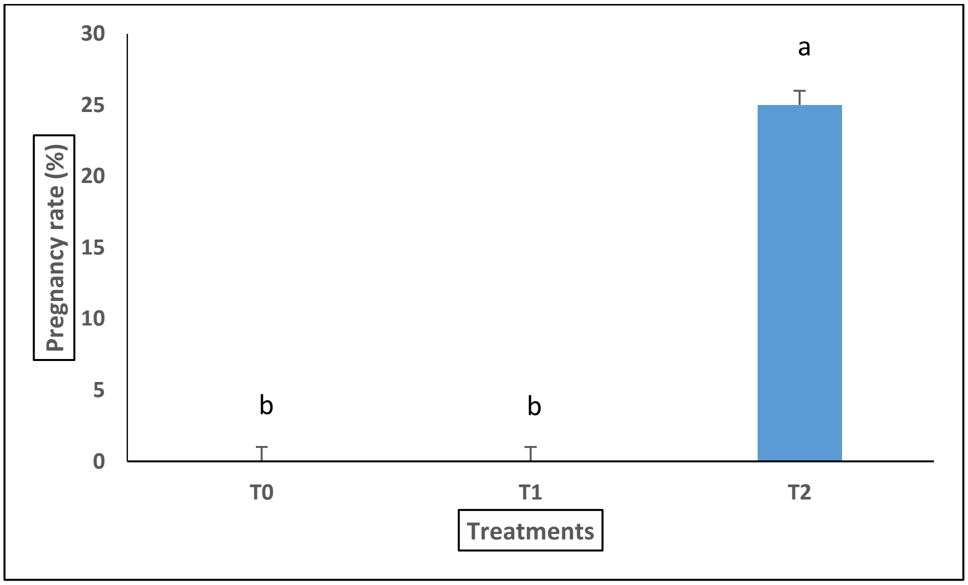


Figure 1: Effect of adding different concentrations of hemp alcoholic extract to Tris-egg yolk extender of local Iraqi goats on the pregnancy rate during the non-breeding season. Lowercase letters significantly differ between treatments. Different treatments were evaluated following two different concentrations of hemp alcoholic extract (T0= control (no treatment), T1=1% 100µL, T2=1% 200µL hemp alcoholic extract).

Regarding fecundity rate, the statistical analysis revealed a significant difference ($P < 0.05$) between T2 ($1 \pm 0.5$) and T1 and T0 ($0.0 \pm 0.0$), respectively, during the non-breeding season (Figure 2).

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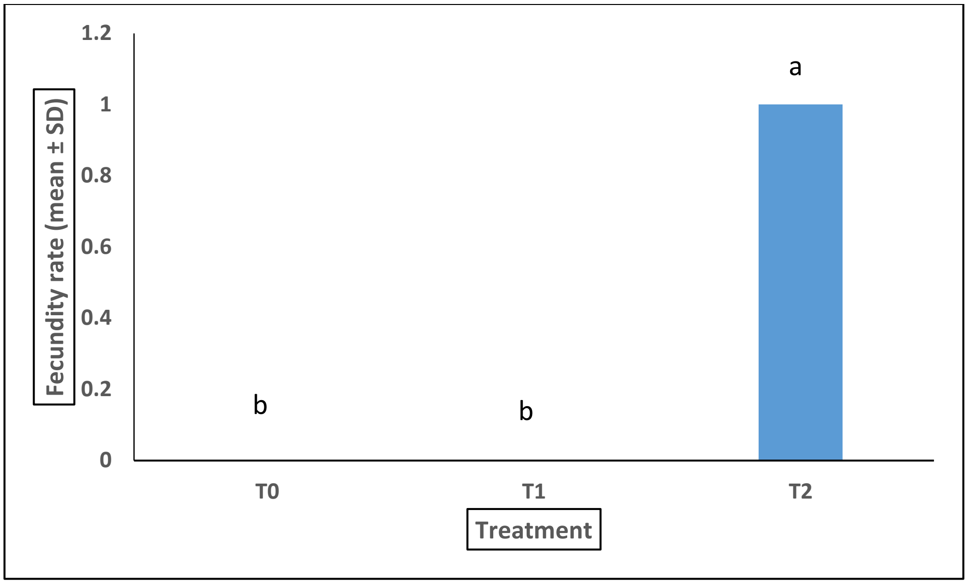


Figure 2 : Effect of adding different concentrations of hemp alcoholic extract to Tris-egg yolk extender of local Iraqi goats on the fecundity rate during the non-breeding season. Lowercase letters significantly differ between treatments. Different treatments were evaluated following two different concentrations of hemp alcoholic extract (T0= control (no treatment), T1=1% 100µL, T2=1% 200µL hemp alcoholic extract).

In the current study, both pregnancy and fecundity rates were significantly higher in 1% hemp alcoholic extract than in other treatments during the non-breeding season. This result was supported by Mehmood et al. (2011), who reported that the pregnancy rate in artificially inseminated goats were 29.4% in the non-breeding season, recorded in goats inseminated with seminal fluid diluted with skimmed milk compared to (12.5%) for goats inseminated with seminal fluid diluted with Tris citric acid [20]. In addition, in equines, hemp supplementation resulted in increased mating behavior, libido, and spermatogenesis parameters [12–14]. Also, Kuzma-Hunt et al. (2024) found that fertilizing bovine ova with hemp-treated sperm did not negatively affect fertilization and embryonic development rates [21]. However, male mice found that chronic ingestion of hemp negatively affected sexual behavior and fertility rates [22]. Our results showed a low pregnancy rate, indicating the effects of heat stress and non-breeding on female reproductive ability and semen

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viability in local Iraqi goats. In addition, the promising low pregnancy rate in T2 (25%) suggests that hemp alcoholic extract may exert a dual effect, acting as an antioxidant that preserves DNA and mitochondrial integrity [10]. However, the failure of T1 (the lower dose) suggests a dose-dependent threshold required to counteract the reactive oxygen species (ROS) generated during the chilling process [23]

It is important to note that the artificial insemination of these Iraqi domestic goats was performed outside the breeding season, consistent with pregnancy rates observed in other non-breeding-season studies. The data are roughly consistent with the results of estrous stimulation using vaginal sponges in temperate [24] and tropical goat breeds [25]. This might be due to heat stress affecting the expression patterns of reproductive-related genes in Malabari goats. A 45-day study using 12 Malabari goats aged within 1 year revealed that heat stress altered gene expression associated with reproductive activity at the uterine level. This alteration may indicate reduced reproductive efficiency in Malabari goats [26]. The egg can also be damaged by heat stress in the early stages of follicular development, and remains sensitive to it in the pre-ovulatory period. Changes in egg quality resulting from heat stress are caused by altered patterns of follicular development, and possibly by direct effects of elevated body temperature on the egg [27].

## conclusion

Finally, although the low fertility outcome following diluted semen treated with hemp alcoholic extract, this result might be a promising outcome to use hemp extract supplementation on goat semen. But these results require additional experiments to account for season, concentration, timing of artificial insemination, and sample size.

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**Conflict of Interest**:

We disclosed that there was no conflict of interest.

## References


(1)**Suwor F, Paengkoum P, Nakharuthai C, Kubota S and Ponchunchoovong S (2025).** Effect of antioxidants on quality and DNA methylation of frozen Anglo-Nubian goat semen Small Rumin. Res. 244 107447.

[2] **AL-Qasimi, R. H., & AL-LAMI, J. S. (2016).** Effect of addition Iraqi probiotic to rations of local goat kids on some cellular and biochemical traits of blood. *Euphrates J. Agric. Sci*, *8*(2), 155-160. [3] Habeeb H and Kutzler M 2021 Estrus Synchronization in the Sheep and Goat *Vet. Clin. North Am. Food Anim. Pract.* **37** 125–37.

[3] **Habeeb H and Kutzler M (2021)** Estrus Synchronization in the Sheep and Goat *Vet. Clin. North Am. Food Anim. Pract.* **37** 125–37.

[4] **Abd A (2025)** Induction of Fertile Estrus During Anestrus Season by Sulpiride in Iraqi Local Goats *J. Res. Vet. Sci.* **4** 99–103.

[5] **Yotov S A, Velislavova D V and Dimova L R (2016)** Pregnancy rate in Bulgarian White milk goats with natural and synchronized estrus after artificial insemination by frozen semen during breeding season *Asian Pacific J. Reprod.* **5** 144–7.

[6] **Zuidema, D., Kerns, K., & Sutovsky, P. (2021).** An exploration of current and perspective semen analysis and sperm selection for livestock artificial insemination. *Animals*, *11*(12), 3563.

[7] **Lin Y, Lv G, Dong H J, Wu D, Tao Z Y, Xu S Y, Che L Q, Fang Z F, Bai S P, Feng B, Li J and Xu X Y (2017)** Effects of the different levels of dietary vitamin D on boar performance and semen quality *Livest. Sci.* **203** 63–8.

[8] **Gao S, Heng N, Liu F, Guo Y, Chen Y, Wang L, Ni H, Sheng X, Wang X, Xing K, Xiao L and Qi X (2021)** Natural astaxanthin enhanced antioxidant capacity and improved semen quality through the MAPK/Nrf2 pathway in aging layer breeder roosters *J. Anim. Sci. Biotechnol.* **12** 1–15.

[9] **Fallahi, S., Bobak, Ł., & Opaliński, S. (2022).** Hemp in animal diets—cannabidiol. *Animals*, *12*(19), 2541.

[10] **Alagbonsi, A. I., Olayaki, L. A., Abdulrahim, H. A., Adetona, T. S., & Akinyemi, G. T. (2019).** Cannabinoid-deficient Benin republic hemp (Cannabis sativa L.) improves semen parameters by reducing prolactin and

enhancing anti-oxidant status. *BMC complementary and alternative medicine*, *19*(1), 132.

[11] **Meador M A, Ates S and Kutzler M A (2024)** Feeding spent hemp biomass does not adversely affect fertility in rams *Am. J. Vet. Res.* **85.**

[12] **Mason A C, Byars P C, Jones T N, Jones B W and Guay K A (2023)** 136 The effects of cannabidiol on libido and heart rate in stallions *J. Equine Vet. Sci.* **124** 104438.

[13] **Byars P C, Mason C, Jones T N, Cassens D and Guay K A (2023**) 135 Effects of oral supplementation of cannabidiol on stallion spermatogenesis *J. Equine Vet. Sci.* **124** 104437.

[14] **Mason A C, Byaers P C, Jones T N, Jones B and Guay K A (2023**) Effects of Oral Supplementation of Cannabidiol on Young Stallion Behavior *J. Anim. Sci.* **101** 27.

[15] **Rossato M, Popa F I, Ferigo M, Clari G and Foresta C (2005)** Human sperm express cannabinoid receptor Cb1, the activation of which inhibits motility, acrosome reaction, and mitochondrial function *J. Clin. Endocrinol. Metab.* **90** 984–91.

[16] **Palade, L. M., Habeanu, M., Marin, D. E., Chedea, V. S., Pistol, G. C., Grosu, I. A., ... & Taranu, I. (2019).** Effect of dietary hemp seed on oxidative status in sows during late gestation and lactation and their offspring. *Animals*, *9*(4), 194.

[17] **Arrebola F A, Torres-Martell R, González-Casquet O, Meza-Herrera C A and Pérez-Marín C C (2022)** Periovulatory Hormonal Profiles after Estrus Induction and Conception Rate by Fixed-Time AI in Payoya Goats during the Anestrous Season *Animals* **12** 2853.

[18] **Habeeb H, Hazzard T, Stormshak F and Kutzler M (2020)** Ovulation, pregnancy, and lambing rates during nonbreeding season with or without exogenous gonadotropin stimulation *Clin. Theriogenology* **12** 23–8.

[19] **SAS (2012)** Statistical Analysis System, User's Guide. Statistical.

[20] **Mehmood A, Andrabi S M H, Anwar M and Rafiq M (2011)** Estrus synchronization and artificial insemination in goats during low breeding season-A preliminary study *Pak. Vet. J.* **31** 157–9.

[21] **Kuzma-Hunt A G, Sabry R, Davis O S, Truong V B, Khokhar J Y and Favetta L A (2024)** THC and sperm: Impact on fertilization capability, pre-implantation in vitro development and epigenetic modifications *PLoS One* **19.**

[22] **Carvalho R K, Rocha T L,**

**Fernandes F H, Gonçalves B B, Souza M R, Araújo A A, Barbosa C C, Silva D M, Campos H M, Tomazett M V., Ghedini P C, Guimarães F S, Andersen M L, Santos F C A and Mazaro-Costa R (2022)** Decreasing sperm quality in mice subjected to chronic cannabidiol exposure: New insights of cannabidiol-mediated male reproductive toxicity *Chem. Biol. Interact.* **351** 109743.

**[23] Al-Rubaie D L, Habeeb H M H and Al-Qasimi R H H (2025)** Effect of Different Levels of Hops (Humulus lupulus L.) Alcoholic Extract on Short-Term Chilled Storage of Local Iraqi Goat Semen With and Without Seminal Plasma Removal *J. Anim. Heal. Prod.* **13** 1073–80.

**[24] Leboeuf B, Forgerit Y, Bernelas D, Pougnard J L, Senty E and Driancourt M A (2003).** Efficacy of two types of vaginal sponges to control onset of oestrus, time of preovulatory LH peak and kidding rate in goats inseminated with variable numbers of spermatozoa *Theriogenology* **60** 1371–8.

**[25] Freitas V J F, Rondina D, Lopes Júnior E S, Teixeira D I A and Paula N R O (2004).** Hormonal treatments for the synchronisation of oestrus in dairy goats raised in the tropics *Reprod. Fertil. Dev.* **16** 415–20.

**[26] Amitha J P, Krishnan G, Bagath M, Sejian V and Bhatta R (2019).** Heat stress impact on the expression patterns of different reproduction related genes in Malabari goats *Theriogenology* **131** 169–76.

**[27] Hansen P J (2019).** Reproductive physiology of the heat-stressed dairy cow: implications for fertility and assisted reproduction *Anim. Reprod.* **16** 497–507.

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