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Effects of menotrophin and chorulon on superovulation in Red Sokoto does

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Abstract

The aim of this study was to compare the efficiency of menotrophin, chorulon and their combinations in superovulation in Red Sokoto does. Fifteen healthy does weighed between 14 and 25 kg were divided into 3 groups of 5 (n=5) each. All does in the 3 groups were synchronized with CIDR for 14 days. Superovulation was performed as follows: Group 1 were treated with 37.5 IU menotrophin for three days, group 2 were treated with 500IU chorulon for 1 day and group 3 were treated with 18.75 iμ menotrophin for 3 days and 250IU chorulon for one day. The mean SEM superovulatory response in group 1, 2 and 3 was (2.6 0.92, 3.2 0.58 and 9.4 0.68) respectively. The superovulatory response showed no significant differences between group 1 and 2 however, there were statistically significance differences ($p<0.01$) between groups 1 and 3 and between 2 and 3. These result showed that the combination of Menotrophin and Chorulon effectively produces multiple ovulations as a result the use this combination is advocated.

Keywords: Chorulon, CIDR, Doe, Menotrophin, Sokoto, Superovulation
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Introduction

Superovulation is an important part of multiple ovulation and embryo transfer (MOET) programme and has the potential to increase the reproductive performance of selected donors and breeds in high demand (Lehloenya *et al*., 2006). The primary goal of super ovulation is to obtain constant high number of viable good quality embryos from each donor (Goal & Agrawal, 2005). Effective ovarian stimulation requires knowledge of the basic concepts of follicular dynamics as well as an understanding of the respective roles of FSH and LH in regulating follicular development and ovulation (Lehloenya *et al*., 2008). FSH stimulates follicle development by interacting with specific receptors on granulosa cells, inducing increased division of granulosa cells and aromatase activity as well as inducing expression of key genes involved in follicle maturation and the formation of LH receptors. Such receptors are essential for completion of follicle maturation and to allow ovulation to occur in response to the preovulatory surge of LH (Lehloenya *et al*., 2008). Superovulation in Does involves the use of FSH, FSH + LH and PMSG (Iheukwumere *et al*., 2008). FSH + LH (Menotrophin) a human menopausal gonadotrophin is one of such preparations that induces ovulation in animals (Abu *et al*., 2008). Human chorionic gonadotropin (hCG) has the α1 subunit in common with the glycoprotein hormones LH, FSH and TSH.

Due to the structural similarity of hCG with LH, it exerts an effect by binding to LH receptors (Saleh *et al.*, 2012). In domestic animals, hCG has a wide range of applications, it is used in the context of estrus synchronization in cattle and horses and ovulation induction in sheep and goats; but also to overcome the negative effect of premature regression of corpora lutea after superovulatory treatment in goats and to improve pregnancy rate in cattle and goats (Saleh *et al.*, 2012). Most super ovulatory treatments are cumbersome, expensive and accompanied by endocrine repercussions that take one or more subsequent cycles to subside (Holtz, 2005). Therefore, several attempts have been made to devise less labor-intensive treatment regimen without compromising oocyte or embryo yield by applying a one shot-treatment regimen consisting of *a* single dose of FSH combined with a moderate dose of eCG (e g., 60-80 mg FSH and 300 IU eCG) (Baldassarre *et al.,* 2007).

There is paucity of information on the use of Menotrophin (FSH + LH) singly and its combination with Chorulon in superovulation on Red Sokoto does in the study area. Therefore this research was aimed at exploiting the potentials of these hormones combination in superovulation.

Materials and Methods

The research was carried out in the small ruminant experimental unit of Veterinary Teaching Hospital Usmanu Danfodiyo University Sokoto, Sokoto state, Nigeria. Sokoto is geographically located in the north western part of Nigeria between longitude 11^0 30' and 13^0 50 East and latitude 4^0 and 6^0 40' N (NPC 2006).

Experimental animals

Sixteen Red Sokoto goats (15 does and 1 buck) weighing between 14-25kg and aged 12-36 months with body condition score 3-4 on a scale of 1-5 (BCS) were used. They were purchased from local markets around Sokto.

The animals were conditioned for 21 days during which they were physically examined and laboratory investigations conducted on the fecal sample for helminthes eggs and blood for hemoparasite and full blood count.

The animals were prophylactically treated with antibiotics (Oxytetracycline 20% w/v Vetindia Pharmaceuticals Limited) and dewormed with (2.5% Albendazole). They were fed wheat bran, bean husk and hay. Water was provided *ad libitum*. Does were synchronized with Controlled internal drug release (CIDR) (Eazi Breed, Newzeland) containing 0.3g progesterone for 14 days as described by Nasroallah *et al.* (2011). CIDR was inserted into vaginal cavity of each doe (on day one), on day 14 the device was removed and signs of estrus were observed.

The does were then randomly divided into 3 groups of 5 does each. The groups were designated as group 1, group 2 and group 3

Superovulation

Group 1 were treated with menotrophin® (75 i.u FSH and 75 i.u LH per ml) as describe by Iheukwumere *et al*. (2008) with slight modification using 37.5 i.u instead of 19.0 i.u used by the previous researchers. Prior to use, the content of the vials of menotrophin® (Samarth life science PVT LTD Mumbai) were dissolved in ml of physiological saline provided resulting in a solution of 75 i.u FSH and 75 i.u LH per ml. Each doe in this group was injected with 37.5 i.u menotrophin for 3 days consecutively.

Group 2 were treated with 500 i.u chorulon® (hCG) (Inter Vet S.A) intramuscularly once as described by Umaru *et al*. (2013).

Group 3 were superovulated using a combination of menotrophin® and chorulon® at the dose rate of 18.75i.u im for three days and 250 i.u im once respectively.

Assessment of superovulatory response

Superovulatory response was assessed by laparotomy based on the methods described by Umaru *et al*. (2013). Briefly, the ventral abdominal region of the does were shaved and scrubbed with chlorhexidine and alcohol. The animals were placed on dorsal recumbency and draped using rectangular draping, 7mg/kg xylocaine was administered subcutaneously based on the weight of each doe. Size 10 scalpel blade was used to make a linear incision from the base of the udder to the umbilicus, a small incision was made on the linear alba with scalpel blade and extended with scissors the ovaries were explored and exteriorized, the number of copora lutea on each ovary (left and right) were counted. The linear alba was then sutured using size 1 poly glycolic acid (PGA) suture material (Wegosutures, foosin medical supplies Inc. LTD Shandong China). The skin was closed using ford interlocking suture pattern with nylon size 0 (Agary Pharmaceutical LTD by Huaiyin medical instrument co., LTD Jiangsu China).

Data on superovulation response were expressed as mean ± SEM and analyzed by post-hoc using the

Table 1: Superovulatory response of RSD treated with menotrophin, chorulon and their combination

Treatment groups	Left Ovary	Right Ovary	Mean ± SEM
Menotrophin (37.5 i.u) (n=5)	3	10	2.6 ± 0.92[a]
Chorulon(500 i.u) (n=5)	8	8	3.2 ± 0.58[a]
Menotrophin(18.75 i.u) + Chorulon (250 i.u) (n=5)	17	30	9.4 ± 0.68[b]

n= number of Animals in each group

Means in the same column with different superscript alphabet differs significantly

tukey test (Graphad instat version 3.0). ANOVA was used to compare means between treatment groups. Values of p≤ 0.05 were considered significant.

Results and Discussion

The estrus response was 100%, time to onset of estrus was 49.0±0.83h and duration of estrus was 72.0 ± 0.89h. Table 1 shows the superovulatory response of menotrophin, chorulon and menotrophin + chorulon. In menotrophin treated group there were 13 ovulations, 3 on the left ovary and 10 on the right ovary (2.6 ± 0.92) while in the chorulon treated group there were 16 ovulations in which 8 were seen on the left ovary and 8 on the right ovary (3.2 ± 0.58). However, the menotrophin + chorulon treated group, 47 ovulations were recorded 17 on the left ovary and 30 on the right ovary (9.4 ± 0.8). Group 3 treated with menotrophin + chorulon had the highest mean superovulatory response of 9.4 copora lutea. The superovulatory response in this group was significantly higher than response of groups 1 and 2 with 2.6 and 3.2 copora lutea respectively ($p < 0.01$). Although group 2 had higher mean superovulatory response (3.2) than group 1, the difference was not statistically significant ($p>0.05$).

One hundred percent of the does treated exhibited standing estrus within 2-3 days post CIDR removal. The high degree of estrus synchrony achieved indicated that the CIDR effectively prolong the luteal phase of the cycle. This percentage response observed in this study is higher than the values of Omontese *et al*. (2010) who reported 20% estrus response with FGA and 55.6% estrus response using CIDR. However it is in agreement with Omontese *et al.* (2014) where 100% estrus response was recorded in ewes treated with CIDR. The onset of estrus recorded in this study is in agreement with that of Omontese *et al*. (2014) who reported 46.1 ± 37.2h in ewe synchronized with CIDR. The findings of our research on the duration of estrus is in agreement with that of Hashemi *et al*. (2006) who reported that the duration of estrus were between 18-72hrs. Oyeyemi *et al.* (2012) reported a shorter duration of 31.11 ± 2.74h in West African dwaf does when compared to the findings of this research. The differences may be as a result of agents used, breeds and environmental condition.

The super ovulation response was low (2.6 ± 0.92; 3.2 ± 0.58) when the does were treated with menotrophin and chrorulon respectively. The low response observed may be due to the failure of the does to respond to the treatment or presence of dominant follicle at the time of superovulatory treatment.

The result of this study agrees with that of Mishra *et al*., (2004), who reported 2.09 ± 0.86 using eCG and hCG and similar to the findings of Mushtaq & Zahida (2010) in which 18 ovulation were recorded in 7 goat treated with 300IU hCG. Esteves *et al*. (2013) also reported similar findings of 1.8 ± 0.6 ovulation using 250IU hCG. Lower Figures for observed parameters were obtained when compared with studies in West African dwaf goat by Iheukwumere *et al*. (2008)

The combination of menotrophin and chorulon effectively superovulated the does in group 3 where a mean of 9.4 was recorded. This might be as a result of the combination of the two agents in which the LH level had been increased due to synergistic effect of LH and hCG which is responsible for maturation and ovulation of the ova. The result is in agreement with that of Lamraoui *et al*. (2014) who reported 10.5 ± 5.4 using PMSG and hCG.

Our findings revealed that super ovulatory hormones used in this study did not effectively produce multiple ovulations when administered singly. However, their combination produced significant multiple ovulations.

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